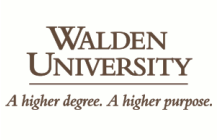

2015

Inflammatory Markers Associated With Disease Progression of Cardiorenal Syndrome

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This is to certify that the doctoral dissertation by

Srikanta Banerjee

has been found to be complete and satisfactory in all respects,
and that any and all revisions required by
the review committee have been made.

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Abstract

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Srikanta Kumar Banerjee

MD, American University of Antigua, 2014

MPH, Des Moines University, 2011

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Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

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Abstract

An increase in cellular inflammatory biomarkers directly increases the risk of cardiovascular disease (CVD). Using the social ecological and biomedical theories, the study examined quantitatively how specific inflammatory biomarkers are associated with cardiorenal syndrome (CRS), a potential complication of hypertension and diabetes, and how sociodemographic factors modify this association in the U.S. adult population. A retrospective secondary data analysis of the data collected from National Health and Nutrition Examination Survey (NHANES) 1999-2010 was utilized to evaluate these hypotheses. High sensitivity C-reactive protein, homocysteine (hcy), and fibrinogen had a modifying effect on Type 4 (chronic reno-cardiac etiology), Type 2 CRS (chronic cardio-renal etiology), and a significant additive effect on CRS even after controlling for known CVD and Chronic Kidney Disease (CKD) risk factors. For Type 4 CRS, the adjusted Odds Ratio of CVD in individuals with CKD was elevated, 2.29 (Confidence Interval [CI] 1.17-3.64, $p < 0.05$), among individuals with elevated hcy levels but close to 1.0 (0.65 CI 0.28-1.53, $p > 0.05$) among patients with normal hcy after the results were controlled for medical and demographic risk factors. Finally, race modified the effect of inflammatory markers on CRS. Out of all the biomarkers, income only modified the effect of hcy on CRS. Education level modified the effect of every inflammatory marker on CRS. While Ferritin-to-Transferrin ratio (F/T ratio) had a non-significant additive effect, due to the lack of adequate subjects, the modifying effect of F/T ratio could not be tested. This study can help initiate social change by urging healthcare professionals to monitor these biomarkers as a part of preventing hypertension, diabetes, and CRS.

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Dedication

I would like to dedicate this to my loving wife Karen Banerjee who has always steadfastly supported me and shared in my hopes and dreams, regardless of the circumstance. She has been a true motivation and inspiration in all of my academic endeavors. I would also like to dedicate this to my late father-in-law and close friend Garry X. Moros who was a true doctor in philosophy at heart and helped me in the pre-planning stages of my dissertation. Through countless philosophical musings and metaphysical discourses, he inspired me and always encouraged me to think outside of the box. My late loving mother-in-law, Joanne Moros' struggle with cardiorenal syndrome inspired me to write this dissertation. She always showed me kindness and support even through the most difficult times. Also, I would like to dedicate this to my cousin and motivator who finally succumbed to complications from cardiorenal syndrome. Finally I would like to dedicate this to my father, Shailendra Banerjee, who introduced me to the concepts of epidemiology.

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Chapter 1: Introduction to the Study

Introduction

Cardiovascular disease (CVD) is the number one cause of mortality and a major public health issue in the United States (Laslett et al., 2012; Roger et al., 2012). Cardiorenal syndrome, a term researchers defined in 2008, refers to an association of closely connected features of bidirectional renal and cardiac dysfunction (Ronco, House, & Happio, 2008; Stucker & Saudan, 2013). Chronic inflammation along with the gradual progression of development and hardening of plaques within the arterial supply leads to CVD (Moore & Tabas, 2010). Chronic inflammatory markers like homocysteine (hcy), Ferritin-to-Transferrin Ratio (F/T), albumin to creatinine ratio (ACR), fibrinogen, and hs-CRP are well-known biomarkers of cardiovascular health (Maurer et al., 2010; Nguyen, Lane, Smith, & Nguyen, 2009; Rosner, Ronco, & Okusa, 2012). Additionally, the high incidence of suboptimal renal function in the elderly population, reaching epidemic levels, is due to chronic inflammation and widespread oxidative stress (Elewa et al., 2012; Vlassara et al., 2009). Due to the established connection between inflammation and cardiovascular disease and chronic kidney disease individually, the link between inflammation and cardiorenal syndrome is hypothesized. The main objective of this dissertation is to evaluate the hypotheses that elevated levels of cellular markers of inflammation, which are caused by diabetes, obesity, smoking, and elevated serum cholesterol levels increase the risk of cardiorenal syndrome, 20 years of age and older. Data from the National Health and Nutrition Examination Survey (NHANES), 1999-2010, was utilized for systematic analysis. The aforementioned NHANES dataset was

analyzed to determine the effect of elevated levels of hcy, F/T, fibrinogen, and hs-CRP on the risk of cardiorenal syndrome.

Statement of the Problem

Cardiorenal syndrome (CRS) is closely related to heart disease and is associated with increased mortality, growing complications, and increased cost of care (Lekawanvijit, Kompa, Wang, Kelly, & Krum, 2012). Cardiorenal syndrome can be defined as a bidirectional pathological impairment of either the heart or the kidney as a result of acute or chronic primary dysfunction in either organ (Stucker & Saudan, 2013). Depending on the primary organ affected and the acuteness of the condition, this syndrome constitutes five subtypes (Ronco, House, & Haapio, 2008). For instance, as many as one-third of individuals who have acute decompensated heart failure have been shown to have type 1 CRS (Ronco et al., 2010). However, little research has been done in studying how to prevent CRS (Lekawanvijit et al., 2012). Understanding the pathophysiology in specific cardiovascular disease like congestive heart failure can lead to identifying critical biomarkers that could signal clinically aberrant manifestations before they occur (Xue, Chan, Sakariya, & Maisel, 2010). Biomarkers are the important solution in slowing or halting cascade and progression of disease development (Wang et al., 2012). More specifically, by becoming aware of any increase in biomarkers (i.e. hs-CRP and hcy) healthcare providers can shift their focus from tertiary prevention to secondary prevention of CRS in populations at high risk (Veerana et al., 2011). Before clinical manifestations of CRS occur, abnormalities at the molecular and the cellular level take place (Ronco et al., 2008). By investigating and addressing the importance of

inflammation as potential biomarkers, public health practitioners and healthcare advocates and providers can effectively empower patients to improve their own health and educate patients about how to control inflammation and slow or prevent disease progression.

In summary, some of the major inflammatory markers have been shown to be closely associated with CRS before the syndrome takes full clinical effect (Veerana et al., 2011), but the problem is that these occurrences have not been demonstrated in a nationally representative multiethnic population (El-Refai et al., 2011). Secondly, the degree to which biomarkers interact has not been established. Hence, the purpose of the study is to clearly delineate the role of inflammation in the development of CRS in a nationally representative multiethnic population.

Nature of the Study

This quantitative study was performed using secondary data analysis with NHANES data from 1999-2010. The analysis provided a quantitative analysis of noninstitutionalized men and women within the general U.S. population and the prevalence of diabetes, obesity, hypertension, smoking status, elevated serum cholesterol levels, and elevated inflammatory markers and their association with CRS (Levitan et al., 2009; Liu et al., 2012; Nguyen et al., 2009). The first two questions addressed the bidirectional impact of inflammatory markers in the specific subtypes CRS. The next question examined whether inflammatory markers have an independent, additive effect on CRS. Finally, the last question addressed the differential impact of race and

socioeconomic status on inflammation and CRS. These four questions were addressed through multiple regressions controlled for confounding factors.

Research Questions and Hypothesis

Research Question 1:

Do elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) modify the effect of CKD on CVD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o1: The elevated inflammatory biomarkers do not modify the effect of CKD on CVD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

H_a1: The elevated inflammatory biomarkers do modify the effect of CKD on CVD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

Research Question 2:

Do elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) modify the effect of CVD on CKD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o2**:** The elevated inflammatory biomarkers do not modify the effect of CVD on CKD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

H_a2: The elevated inflammatory biomarkers do modify the effect of CVD on CKD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

Research Question 3:

Do elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) have an additive effect on CRS along with CVD risk factors (e.g. obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o3: Elevated inflammatory biomarkers do not act as additive risk factors to CRS along with known CVD risk factors.

H_a3: Elevated specific inflammatory biomarkers do act as additive risk factors to CRS along with known CVD risk factors.

Research Question 4:

Do sociodemographic (race/ethnicity, family income, expressed relative to the poverty threshold, or education level) indicators play a modifying role between the relationship of inflammatory markers and CRS controlling for known CVD risk factors (e.g. obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status?

H_o4: Sociodemographic indicators do not play a modifying role between the relationship of inflammatory markers and CRS.

H_a4: Sociodemographic indicators do play a modifying role between the relationship of inflammatory markers and CRS.

Purpose of the Study

While researchers have explored certain subsets of CRS in African American demographic populations previously (Lea et al., 2009), inflammatory biomarkers in the context of the development of cardiovascular disease and the subsequent development of chronic kidney disease or vice versa among a multi-ethnic high risk population group have not been explored, studied, or investigated adequately in the past. This study may provide public health practitioners with early detection tools that can be specifically geared towards certain populations that are in the highest risk for CRS. Insights gained from this study will reduce long-term healthcare costs by aiding public health practitioners, healthcare providers, and healthcare advocates to make a positive difference in the health and quality of life in high-risk individuals who have early or preliminary cardiovascular disease. Secondarily, because the link between inflammation and CRS has been established, the next step is to empower patients through knowledge of cardiorenal disease likelihood in order to achieve health equity in populations with barriers to access**.** Mogford, Gould, and Devoght (2011) found that by motivating, engaging, and empowering individuals on specific health topics like the importance of inflammation in disease progression, improvements can be made in physical health in high risk groups. Through this dissertation, the assertion may be made for a greater awareness of the role of inflammation in disease progression which may help both health practitioners and patients alike. In this medical paradigm shift, treatment changes from focus on each individual organ to treating the body as a whole (Wallace & Wallace, 2004). In cardiovascular disease, health practitioners are beginning to recognize the need

to track inflammatory markers. In a meta-analysis of 52 studies, Kaptoge et al. (2012) found that if inflammatory biomarkers like fibrinogen and hs-CRP are measured in those deemed to have intermediate risk of cardiovascular disease, then a considerable amount of subsequent cardiovascular events can be decreased. Similarly, the importance of inflammatory markers in the development of renal disease needs to be emphasized among patients and become a public health priority (Stenvinkel, 2010). By investigating and addressing the possible importance of inflammation as potential biomarkers, public health practitioners may implement social change by empowering patients to improve their health and teaching patients to control inflammation effectively and slow or prevent disease progression.

Theoretical Framework

The primary theoretical framework for this study was the biomedical theory of disease. This theoretical framework suggests CRS is a consequence of biological aberrations in the body. More specifically, biomarkers can be used as an early marker for identifying the eventual consequence of CRS (Mayeaux, 2004). Additionally, the Bronfenbrenner's social ecological model indicates that the nature of the community in addition to individual demographic indicators like race, gender, and socioeconomic status makes a difference in health status (Ceci, 2006; Henry, Donna, Jennifer, & Colleen, 2012). From this theory, it follows that the inflammatory markers might be different according to the sociodemographic group and place of residence. Additionally, the social ecological model purports that there are complex social determinants of health like the interplay of community, interpersonal, societal, and personal interactions that impact

cardiovascular risk factors (Chatterji, Joo, & Lahiri, 2013; Fleming & George, 2008). Extrapolating from this theory, CRS would have a similar impact from social determinants of health.

Conceptual and Operational Definitions

The following section is composed of different conceptual definitions of specific terms that are used throughout the study. The conceptual and operational definitions are both included in this section. The purpose of the conceptual definitions is to explain what the connotation of each term is in the context of this study, and the purpose of the operational definition is to demonstrate the empirical context of the term. While some of the conceptual definitions were included in this section, the vast majority of definitions have been further delineated in detail in Chapter 3. In contrast to conceptual definitions, the operational definitions were empirically derived from definitions found in the literature.

Body mass index (BMI): The BMI is calculated and operationalized through weight (kg) divided by height (m^2) (Eknoyan, 2008). BMI is a standardized unit that takes into account body shape and used as an index of obesity.

C-reactive protein (CRP): An acute phase pentameric protein found on chromosome 1 and produced primarily by the liver (Yeh, 2005). However, renal, respiratory epithelial cells, and coronary artery smooth muscle cells have been shown to produce this protein as well. Chronic inflammation and CVD have been associated with elevated levels of CRP (Razavi et al., 2013). In this study CRP was quantified by hs-

RP. The hs-CRP is a highly sensitive laser nephelometry test that is used to measure the pertinent deviations.

Cardiorenal syndrome (CRS): Cardiorenal syndrome is a well-recognized recently classified syndrome, in which healthcare professionals have understood that prognosis is much worse when renal dysfunction occurs simultaneously with cardiac dysfunction (McCullough & Ahmad, 2011). The symptoms that ensue are part of this syndrome.

Diabetes: A disorder that is characterized by insulin resistance or autoimmune destruction of beta cells in the pancreas (McPhee, Papadakis, & Tierney, 2013).

Ferritin-to-transferrin receptor ratio: Ferritin is a nanocage structured acute phase protein, which store 4,500 Fe (III), and increased amounts can have been associated with chronic inflammatory conditions like Rheumatoid Arthritis (Vanarsa et al., 2012). The purpose is to prevent the infective agent from attaining access to iron. A ferritin based inflammatory marker controlling for status of iron is the purpose of the F/T ratio (Skinner, Steiner, Henderson, & Perrin, 2010). Because there are no specific clinical cutoffs that actually are present for this measure, in previous studies the defined F/T saturation was considered high when the value was greater than the weighted 95th percentile in the final analytic sample (Skinner et al., 2010).

Fibrinogen: Fibrinogen is a 340 kDa plasma glycoprotein that is converted to fibrin and associated with systemic inflammation (Ford, 2003).

Homocysteine: This is an amino acid which increases with inflammation, associated with increased oxidative stress, and has been shown to increase the risk of CVD (Oudi et al., 2010).

Obesity: A condition that is characterized by an increased number of adipose tissue and operationalized by a BMI ≥30 kg/m^2 (McPhee, Pappadakis, & Tierney, 2013). Obesity is associated with increase inflammatory markers and metabolic syndrome (McPhee et al., 2013).

Assumptions, Advantages, Limitations, and Delimitations

Assumptions

Multiple assumptions were made based on previously published research for the purposes of this study. The different inflammatory markers hcy, F/T, fibrinogen, and hs-CRP have individually been demonstrated to be associated with higher frequency of cardiovascular disease. The two inflammatory markers hs-CRP and hcy have received so much attention, that some researchers have either recommended or integrated these markers into risk scoring systems for predicting 10-year CVD risk (Maurer et al., 2010; Rosner, Ronco, & Okusa, 2012). Another assumption is that by integrating different frameworks, a unique model can be ascertained to find the association between inflammation and CRS. Next, the assumption can be made that that ferritin-to-transferrin ratio can be used as an inflammatory marker because inflammatory outcome which controls for status of iron (Skinner, Steiner, Henderson, & Perrin, 2010). Additionally, the assumption was made that the responses reported by subjects through the questionnaire were made accurately. Finally, the cardiovascular risk factors tested with CRS are also risk factors for each of the inflammatory markers.

Advantages

There were multiple advantages of this study. The main advantage of these data was that the dataset used included some of the most recent data. Additionally, the sample selected is systematic and nationally representative data. Because the NHANES data have been collected for forty-four years, its reliability is enhanced (CDC, 2013b). Many public decisions are made based on conclusions from these data. Additionally, the laboratory methods used are standardized for each specimen, contributing to the objectivity and the validity of the study.

Limitations

Multiple limitations in this study needed to be adequately addressed. There was insufficient preexisting information concerning the association of inflammatory markers and CRS. Due to this lack of information, a methodical approach was employed where theoretical frameworks and conceptual models were utilized to better understand the association. By applying existing models to better understand the relationship, the etiology of CRS was better elucidated, making prevention a possible option.

The second limitation is that when using previously collected survey data, there are limitations as to what types of questions and how they are asked. When using previously collected data, the problem of the increased statistical error may occur because of the lack of ability to check for complete accuracy of the data presented. Because the NHANES survey has gone through numerous iterations in the past four decades, researchers have included more up to date questions which were adequately designed to accommodate changes to meet stakeholders' needs (CDC, 2013b). Additionally,

due to the inability to check accuracy of data, there may be an increase in statistical error. As mentioned previously, because of the reputation for validity in collection and recording of the data, this potential error is minimal. Any possible errors can also be mitigated by using overlapping information so that data can be cross-checked. Another potential source for error was the reliance on self-reported information, which may lead to bias. Again, in order to address this limitation, objective, examination information was used. While the NHANES dataset is compatible with specific programs, all functions are not available without the knowledge or access to specific programs. Consequently, another limitation is that rudimentary knowledge in weighting methods and analysis in specific statistical software is necessary for the proper use of the data. Finally, a limitation that is an aspect of many cross-sectional studies is that the biomarker levels are a reflection of one moment in time and could be as a result of a spurious factor like infection. To control for these factors a large, a representative population was utilized for this study.

Scope and Delimitations

In this study, only adults aged 20 years or older were included the participant population. This criterion was necessary because the inclusion of minor participants would have added confounding factors that would have influenced the outcome. Additionally, complete information on individual cellular biomarkers for inflammation was necessary. Only subjects who were tested for specific biomarkers were included, when testing the effect of that biomarker on CRS. Furthermore, those subjects who have complete information on BMI, diabetes, serum cholesterol level, and smoking status were

included. Details on the method of statistical analysis were identified prior to the actual data analysis in Chapter 3. This was to make certain that the scope of the study is not crossed (Frankfort-Nachmias & Nachmias, 2008).

Study Significance and Social Change

In the context of inflammation, the conclusions from this study can be used for advancements in the field and social change. This study provides both professional-level data collection and unanalyzed data for future research and social change. The conclusions of this study have overarching implications to implement primary prevention for CRS. Monitoring C-reactive protein and other inflammatory markers, as a method of primary prevention, is another change in clinical practice that can save money and lives in the future for not only CVD, but also in the context of CRS (Yousuf et al., 2013). Despite clinical evidence and creators of certain risk scores, like the Reynolds risk score, having demonstrated the need to integrate the regular use of hs-CRP, this inflammatory marker is still not being standardly recommended by the American Heart Association or the American College of Cardiologists according to the revised guidelines released in November 2013 (Goff et al., 2013; Windgassen, Funtowicz, Lunsford, Harris, & Mulvagh, 2011). Other inflammatory markers are not recommended as frequently for cardiovascular disease. Extrapolating the same associations to CRS, demonstrates the expanded need to universally screen inflammatory markers so that terminal illnesses like acute coronary syndrome and Stage 4 CKD can be prevented. The conclusions from this research have the potential to address the gaps and give proponents additional evidence to make inflammatory

markers a national priority among all organizations. Additionally, making this a national goal like through Healthy People 2020 can let patients and healthcare providers realize the importance of inflammation. Obesity has been associated with inflammation and is an additional reason for a renewed campaign against obesity in the context of CRS (McManus et al., 2013). While Healthy People 2020 has cardiovascular-related goals, there are no goals specifically related to inflammation (Henderson, 2013). A strong argument must be developed in order to convince public policy makers to invest in related research and public health initiatives.

Creating other sources of positive social change within groups at risk of CRS is a very important aspect of this study as well. Analysis of the NHANES survey data provided evidence related to the association between metabolic syndrome, inflammatory markers, and CRS in the context of differential sociodemographic factors within the noninstitutionalized, civilian U.S. population ages 20 years and older. In order to control chronic diseases, the results can be useful for developing and implementing new policies and procedures in target populations. These new policies and procedures can influence additional federal and state funding for interventional programs geared toward combating obesity in specific racial and socioeconomic groups (Henderson, 2013).

Summary

Cardiorenal syndrome is a significant public health concern in the United States and worldwide. While the association between obesity, smoking status, hyperlipidemia, hypertension, and diabetes and CRS has been established, the potential modifying effect of inflammatory markers between the aforementioned variables and CRS has not

been previously established. The intention of the present study was to examine and analyze the representative sample from the general U.S. population for inflammation as independent and additive risk factors for CRS. I was able to identify specific risk factors for CRS and to increase awareness of how inflammation plays a role in the pathophysiology of disease. Chapter 2 of the dissertation will provide a general overview of CVD, CKD, the association of inflammation with CRS, and the possible mechanism and etiology of CRS. Publicly available data were utilized from the National Health and Nutritional Examination Survey (NHANES) that was collected between the years of 1999-2010. Chapter 3 will detail the quantitative research method, the study population, the process of how the data were collected and analyzed. In Chapter 4, the outcomes from univariate, bivariate, and multivariate analyses will be discussed through charts and graphs. Chapter 5 will provide a discussion of the outcomes and the recommendations pertaining to the association between elevated inflammatory markers and CRS.

Chapter 2: Literature Review

Introduction

The primary purpose of this literature review is to assemble systematically all of the current literature regarding the pathophysiological understanding of the development of cardiovascular disease (CVD) and chronic kidney disease (CKD), risk factors, how inflammation plays a role in each condition individually, and the scientific connection between inflammatory biomarkers and cardiorenal syndrome (CRS). In the literature review I explain the pathophysiology of atherosclerosis, the public health burden of CVD, and the connection of inflammation with CVD. In the review I then focus on the public health burden of chronic kidney disease and the connection with inflammation. Finally, I discuss the epidemiology and connection of inflammatory biomarkers to CRS.

Literature Review

An exhaustive literature review was performed in order to compile and analyze previous research. The literature was searched using the following databases: PubMed, ProQuest Central, Science Direct, and the Johns Hopkins intranet. Keywords, individually and in combination, were used to search the literature and included: “CRS”; “cardiorenal syndrome”; "hs-CRP”; “C-reactive protein”; “homocysteine”; “ferritin”; “metabolic syndrome”; “cardiovascular disease”; “serum”; “biomarkers”; “obesity”; “overweight”; “physical inactivity”; “GFR”; “chronic kidney disease”; “cholesterol”; “inflammation”; “gender”; “race”; and “NHANES”. The first authors of many of the articles were contacted about the articles that they wrote. The years were from 1970-2014. The earlier dates were included for historical development of the databases.

Theoretical Foundation

Control of modifiable risk factors like diet, high blood pressure, hypercholesterolemia, tobacco use, weight management, and diabetes has been traditionally targeted to make changes in cardiovascular disease. However, controlling inflammation can have equally beneficial effects (Dzau et al., 2006). The theory outlined below is the biomedical theory of disease used in CVD in which scholars utilize a continuum approach.

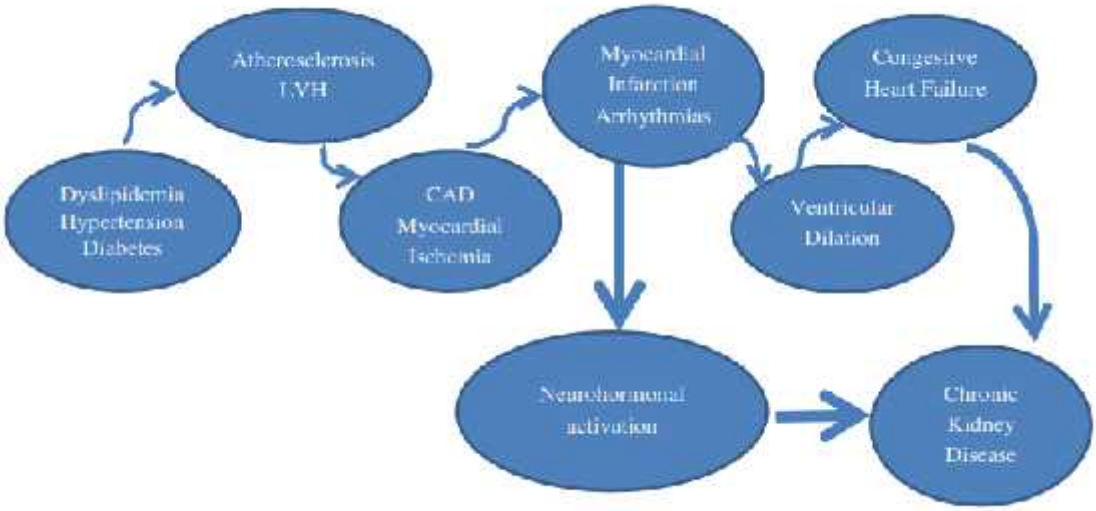

Figure 1. Cardiovascular disease model in the context of atherosclerotic continuum. Adapted from "The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature," by M. Rourke, M. Safar, and V. Dzau, 2010, *Vascular Medicine, 15*(6), 461-468.

Understanding cardiovascular disease sequelae in the context of a continuum is imperative (O'Rourke, Safar, & Dzau, 2010). In the process of understanding the atherosclerotic continuum, the first stage takes place due to the risk factors of hypertension, dyslipidemia, diabetes, smoking, and obesity as outlined in Figure 1. In each of these stages, inflammatory markers play a critical role in exacerbating the symptoms of the disease. These risk factors along with chronic inflammation like hs-CRP released from the endothelial cells can additionally cause atherosclerosis and in turn left ventricular hypertrophy. The next stage is coronary artery disease, which is also interrelated to hs-CRP released from the coronary vessels (Yeh, 2005). Due to coronary artery disease, there is myocardial ischemia and angina (Dzau, 2005). When myocardial ischemia becomes more severe, this becomes coronary thrombosis. Thrombosis can lead to myocardial infarction and acute coronary syndrome. This can lead to arrhythmias and remodeling of the heart. Remodeling leads to ventricular dilatation and congestive heart failure (CHF). CHF leads to end stage heart disease and death. Additionally, CHF is directly connected with CKD.

Additionally, the social ecological model from Bronfenbrenner's ecological systems theory indicates that not only the individual demographic indicators like race, gender, and socioeconomic status, but other demographic factors like the nature of the community makes a difference in health status (Ceci, 2006; Henry, Donna, Jennifer, & Colleen, 2012). From this theory, it follows that the inflammatory markers might be different according to the sociodemographic group and place of residence. Additionally, the social ecological model purports that there are complex social determinants of health

like the interplay of community, interpersonal, societal, and personal interactions that impact cardiovascular risk factors (Chatterji, Joo, & Lahiri, 2013; Fleming & George, 2008). Extrapolating from this theory, CRS would have a similar impact from social determinants of health. Different social determinants in the may play a varying role in the context of CRS.

Cardiovascular Disease

Cardiovascular Disease Definition and Epidemiology

Cardiovascular disease is the primary cause of death of adults in the United States and worldwide (Laslett et al., 2012). Because cardiac dysfunction is closely connected with the renal system, dysfunction in one organ is closely connected with dysfunction in another organ. Early identification of underlying sources of pathology like atherosclerosis and inflammation is imperative. These public health issues are tantamount in importance to the issue of CVD itself. More specifically the category of CVD includes coronary heart disease (i.e. angina, acute myocardial infarction), heart failure, cardiomyopathy, atrial fibrillation, cerebrovascular disease (i.e. stroke), hypertensive disease, peripheral arterial disease, and congenital malformations. Chronic inflammation along with the gradual progression of development and hardening of plaques within the arterial supply leads to CVD.

Cardiovascular disease is an enormous public health burden and a large source morbidity and mortality. In 2008, over 82 million people were estimated to have CVD which led to $180 billion in direct health care expenditures (NIH, 2012). The American Heart Association estimated that between 2010 and 2030 healthcare costs related to CVD

will triple from $272.5 billion to $818.1 billion (Heidenrich et al., 2011). Even though CVD related death rates had dropped by 2-5% each year, in 2008 there were still over 700,000 CVD related deaths, primarily from heart attacks and strokes. The gradual development of atherosclerosis has been established as a source of CVD. Atherosclerosis develops from the combination of benign fatty streaks and intimal thickening during childhood (Spagnoli, Bonanno, Sangiorgi, & Mauriello, 2007). Next there is everything from accumulation of fibrous plaques to accumulation of cholesterol and fat. Finally, the multiplication and migration of smooth muscle cells play a role to solidify the plaques and narrow the lumen of arteries. As an individual ages, stenosis and loss of arterial elasticity leads to lower blood flow and increased chance of plaque rupture creating an embolus. At an average heart rate of 70 beats per minute, the expansion repeatedly stretches the aorta and the connected elastic arteries 30 million times per year (O'Rourke, Safar, & Dzau, 2010). The elastic lamellae frays and fractures, consequently causing distension, remodeling, and stiffening of the aorta and arteries. Because the elastic artery stiffens, the microvasculature experiences shear force and endothelial damage. If this occurs in the kidneys, then the microinfarcts can cause pulse wave nephropathy (Dzau, 2005).

Congestive Heart Failure

Congestive heart failure is difficult to quantify and has many levels to the clinical definition and is therefore subjective. The New York Heart Association utilized Class I-IV in the functional definition (Christensen et al., 2013; Lee et al., 2002). Class I states that the individuals has no limitations to physical activity and does not experience any

angina, fatigue, palpitations, or dyspnea. In Class II, while there is slight limitation of physical activity, the person is comfortable during rest (Christensen et al., 2013). However, the angina, fatigue, palpitations, or dyspnea is present with just ordinary activity. In class III, there significant limitation of physical activity, but still the person is comfortable at rest. In this class patients experience angina, fatigue, palpitations, or dyspnea in less than ordinary activity. Class III is divided into a and b, where IIIa is no dyspnea at rest while IIIb happens when there is recent dyspnea at rest (Lee et al., 2002). Finally, class IV is comprised of patients who are unable to perform physical activity because of excessive discomfort (Williams, Weiss, Patel, Nwakanma, & Patel, 2007). The symptoms are present with activity as well as rest.

Inflammatory Hypothesis of Atherosclerosis

Although atherosclerosis plays a role in CVD progression, certain individuals do not develop active CVD even in the presence of plaques (Moore & Tabas, 2010). Researchers have found that plaques can be either stable or unstable. While stable plaques are slow growing and benign, unstable plaques are necrotic and have many signs of inflammation leading to serious clinical manifestations (Lichtman, Binder, Tsimikas, & Witztum, 2013). More specifically, the atheromatous fibrous cap has a large necrotic core with large inflammatory cells (i.e. macrophages and T-lymphocytes) which lead to an excessively calcified lesion or a complicated hemorrhagic lesion. From this fact follows the need to regularly monitor inflammatory biomarkers. Certain health practitioners have called for the monitoring of inflammatory biomarkers in every patient (Ben-Yehuda, 2007).

One of the first markers, that was an acute phase biomarker known since to rheumatologists since the 1930's was hs-CRP (Deron, 2004). More recently, this pentameric biomarker, produced in the liver, was connected to unstable angina and became a part of guidelines to diagnose and treat acute coronary syndrome (Berk, Weintraub, & Alexander, 1990; Liuzzo et al., 1994; Rapezzi, Biagini, & Branzi, 2008). Researchers that added more evidence to provide overwhelming credibility to the inflammatory hypothesis of CVD and published in *The New England Journal of Medicine*, studied the effect of lipid-lowering drugs on hs-CRP (Nissen et al., 2005; Ridker et al., 2005). Nissen et al. (2005) performed intravascular ultrasound in 502 in order to study the effect of atorvastatin on atherogenesis, LDL, and hs-CRP. They found that after 1.5 years, not only was there slower rate of atherogenesis, but also a reversal of LDL and hs-CRP. Similarly, Ridker et al. (2005) found that individuals who had a lower hs-CRP had more improved clinical outcomes compared to individuals with high hs-CRP.

Numerous clinical trials provided support to the inflammatory hypothesis of CVD, and the importance of screening for hs-CRP in high risk individuals for primary prevention (Kjekshus et al., 2007; McMurray et al., 2009; Mora, Musunuru, & Blumenthal, 2009; Ridker et al., 2008). The Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) and Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA) trials were the classic trials that demonstrated the power of assessing hs-CRP as a primary prevention tool (Ridker et al., 2008). Rosuvastatin is a potent 3-hydroxy-3-methylgluytaryl-CoA

(HMG-CoA) reductase inhibitor which decreases levels of LDL. Researchers from the JUPITER trial found that in a cohort of 17,802 subjects with low-to-normal levels of LDL and elevated levels of hs-CRP > 2 there was a higher reduction in both of these values than in placebo. Furthermore, there was a decrease in one-year the absolute risk stroke, myocardial infarction, and mortality. In another Danish study, Correale et al. (2011) found that the use of statins blunted the inflammatory markers and ameliorated left ventricular function by Doppler studies. Additionally, when a retrospective analysis was performed on the CORONA trial, researchers found that patients on rosuvastatin therapy and those with ≥ 2 mg/L hs-CRP had significantly better cardiovascular outcomes than those with elevated hs-CRP and not on therapy (McMurray et al., 2009). While the JUPITER trial prospectively demonstrated that reduction in one inflammatory marker along with lipid levels lead to a decrease in cardiovascular disease incidence, other randomized control trials are needed to demonstrate that anti-inflammatory drugs alone has an effect to decrease CVD. Consequently, researchers from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) are researching the effect of a monoclonal antibody that exclusively targets inflammation (Ridker, Thuren, Zalewski, & Libby, 2011).

Canakinumab is a potent inhibitor of an inflammatory cytokine interleukin-1β. In a double-blinded control trial, Ridker et al. (2011) allocated randomly 17,200 stable subjects that had elevated hs-CRP and suffered a previous myocardial infarction (MI) to the treatment group who were administered canakinumab versus a control group who were administered placebo. The treatment group was administered 50, 150, or 300 mg of

medication every 3 months. Additionally, researchers from the Cardiovascular Inflammation Reduction Trial (CIRT) trial are studying if methotrexate reduces death, stroke, or MI in those people who have suffered a previous MI and have metabolic syndrome and type 2 diabetes. The results to both of these aforementioned studies are still pending (Ridker, 2009).

Connection of Autoimmune Diseases and Cardiovascular Disease

Another aspect that can serve to support the inflammatory hypothesis of CVD is the connection of autoimmune disease and higher incidence of CVD. Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis are the two primary autoimmune conditions which afflict people and coexist simultaneously to the point that this is known as *rhupus* syndrome (Tani et al., 2013). In fact individuals who have autoimmune diseases with elevated hs-CRP like in SLE invariably suffer from premature mortality due to cardiovascular disease (Gustaffson et al., 2009). This association is important to focus on because it supports the theory that inflammation leads to cardiovascular disease. Additionally researchers found that there was an increased risk of cardiovascular disease due to the inflammation associated with rheumatoid arthritis and that more research needs to focus on risk factors rather than treat these variables as covariates (Liao and Solomon, 2013).

Renal Dysfunction

Description and Epidemiology of Kidney Dysfunction

Renal dysfunction is a major component of the CRS sequelae. Out of individuals who are on dialysis, 33% suffer from heart failure (Stucker & Saudan, 2013). Renal

dysfunction can be divided into two categories—acute and chronic. Acute kidney injury (AKI) is a constellation of signs which are collectively placed together to connote the deterioration of renal function. The AKI is a specific syndrome which includes acute tubular necrosis, acute vasculitic and glomerular renal diseases, acute interstitial nephritis, prerenal azotemia, acute postrenal obstructive nephropathy. According to Marrenzi et al. (2013), not only can Acute Coronary Syndrome (ACS) cause AKI, but the former can be considered as serious as the latter. As noted, the etiology for AKI syndrome can be either from direct renal injury or functional impairment (Van Biesen, Vanholder, & Lameire, 2006). Worsening Renal Failure (WRF) is a traditional way to determine if there is AKI by assessing the change in creatinine from baseline (Di Tano, Misuraca, Ronco, Zocali, & Frigerio, 2006). If the change is greater than or equal to change in 0.3, then this is defined as WRF (Raichlin et al., 2013).

Unlike AKI, chronic kidney disease takes place over an extended period of time and is measured by GFR (Parmar, 2012). Stage 1 CKD is considered to be a GFR $\geq$ 90 mL/min/1.73m^2. Next, stage 2 CKD is considered between 60-89 mL/min/1.73m^2. During this stage there is initial glomerular damage. In stage 3 (30-59 mL/min/1.73m^2) CKD and stage 4 (15-29 mL/min/1.73m^2) CKD, there is fibrosis and sclerosis of the kidneys (Levey et al., 2011). Finally in stage 5 (<15 mL/min/1.73m^2), the patient needs dialysis. There has been considerable controversy as to how to quantify GFR using either creatinine or Cystatin C (Lopes et al., 2013). Additionally, there are different equations to calculate GFR. The two ways that have been compared recently are the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology

Collaboration (CKD-EPI) method of calculation (Masson et al., 2013). According to Shafi et al. (2012), CKD-EPI had better risk stratification than the MDRD method as applied to NHANES III data. CKD has significant detrimental effects on the heart due to the excess sodium and water retention, uremic solute retention, calcium and phosphorus abnormalities, and anemia. In recent years, due to these observed connections the therapeutic effects of endothelin antagonists, adenosine antagonists, B-type natriuretic peptide (BNP) derivative, and vasopressin antagonists have been tested through randomized controlled trials (Ahmed, Wong & Pai, 2010) .

Chronic Kidney Disease and Inflammation

In individuals with CKD, one of the largest causes of mortality is CVD (Go, Chertow, Fan, McCulloch, & Hsu, 2004; McCullough et al., 2007). According to Vlassara et al. (2009), suboptimal renal function in the elderly population is reaching epidemic levels because of chronic inflammation and widespread oxidative stress. In an aging Bavarian population, increased hs-CRP levels and CKD at baseline were associated with a greater prevalence and vascular related diseases (Jalal, Chonchol, Etgen, & Sander, 2011). In a multicenter study in Taiwan, researchers evaluated 22,043 adults for the relationship between hcy and hs-CRP and CKD (Chuang et al., 2013). According to this study, females had plasma hcy that was associated with CKD. However, in males only plasma hs-CRP was associated with CKD.

Inflammation and CRS

Inflammation plays not only a critical role in the progression of CRS, but also in the severity and poor clinical outcome in both CKD and CRS. Inflammatory biomarkers

are helpful in testing and following in assessing the progression of CRS. Researchers have found that hcy, F/T, fibrinogen, and hs-CRP are critical biomarkers that are helpful in (Maurer et al., 2010; Rosner, Ronco, & Okusa, 2012). According to Maurer et al. (2010), from analysis of 161 patients, individuals with high hcy with ≥20 micromol had a higher likelihood of decompensated CHF and death than normal hcy. In this same study, patients with CRS were 3.7 times more likely to have an adverse clinical outcome than those that do not have the syndrome. Additionally, researchers have studied ferritin not only in the context of iron status, but also in the context of inflammation and autoimmune conditions (Claes, Ellis, Rettie, Butcher, & Bradley, 2013; Pan & Jackson, 2008; Vanarsa et al., 2012). In order to control for inflammation, researchers performed a Ferritin/Transferrin (F/T) saturation ratio and F/T was considered elevated if it was >4.81 (Skinner, Steiner, Henderson, & Perrin, 2010). The ratio was better because it controlled for iron status.

Another key inflammatory marker that researchers have traditionally associated with cardiovascular disease is hcy (Davalos & Akassoglou, 2012; Ridker, Hennekens, Buring, & Rifai, 2000). Homocysteine has been demonstrated to increase free radical activity and oxidative stress (Jonasson, Ohlin, Gottsäter, Hultberg, & Ohlin, 2005). This action aids in the oxidizing of low-density lipoprotein (LDL) inducing atherosclerosis. Furthermore, hcy promotes the synthesis of other inflammatory cytokines in smooth muscle and endothelial cells.

CRS Definition

In order to investigate CRS, the first step researchers had to take was to define and elucidate what CRS entailed. In 1951, the term CRS was first coined in France to mean the simultaneous failure of the heart and kidney (McCullough et al., 2013). However, until recently, the definition and the classification were unclear. In 2004, The National Heart, Lung, and Blood Institute took a stance in a report by loosely defining CRS as a hindrance to treatment of CHF. The decline in the function of the kidneys reflected by decreasing GFR was seen as a contributory factor for why therapy against congestive symptoms failed against CHF. In September 2008, Ronco and colleagues (2008) participated in a consensus conference composed of nephrologists and intensivists known as the Acute Dialysis Quality Initiative (ADQI), and defined CRS and concretely identified five subtypes. They defined CRS as an inclusive term identifying a disorder, "whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ" (Ronco et al., 2008). Different authors have different perspectives on the proper definition, but much of the confusion was clarified through a subsequent ADQI which was held in November 2012 (Hasse et al., 2013).

At the eleventh ADQI conference, in Venice, Italy, the pathophysiology was updated and more researchers defined CRS, resolving to spread more awareness in this relatively novel model (McCullough et al., 2013). However there is still debate about the exact mechanism and pathophysiology of CRS and more is being done to find how to effectively treat this syndrome at an early stage. Different organizations are placing efforts to make a more collaborative effort to create an interdisciplinary to explore this

difficult topic. In 2011, the Cardiorenal Society of America (CSRA) was created to increase awareness about this syndrome (CSRA, 2011). They meet annually to discuss about research and clinical progress that scientists have made in the previous year. More effort and non-profit organizations need to place more effort in trying to prevent CRS in those individuals with underlying CKD or CVD. With the collaboration of physicians and health care workers in both disciplines, more important and awareness trickle down to patients as well. The different subtypes are clearly illustrated in Figure 2 (McCullough, 2010). Type 1 CRS entails patients who had kidney dysfunction or injury due to a primary acute deterioration of heart function (Hasse et al., 2013; Ronco, Cicoira, & McCullough, 2012). As McCullough (2010) demonstrates in the figure, acute coronary syndrome, acute decompensated heart failure, and even contrast procedures can cause a domino effect from the heart to the kidneys. Increased central venous pressure and renal congestion along with oxidative stress causes irreversible necrosis and apoptosis of nephrons in the kidneys. More importantly, cytokines are involved in this process as well, which are closely interrelated with hs-CRP (Papadakis et al., 2013).

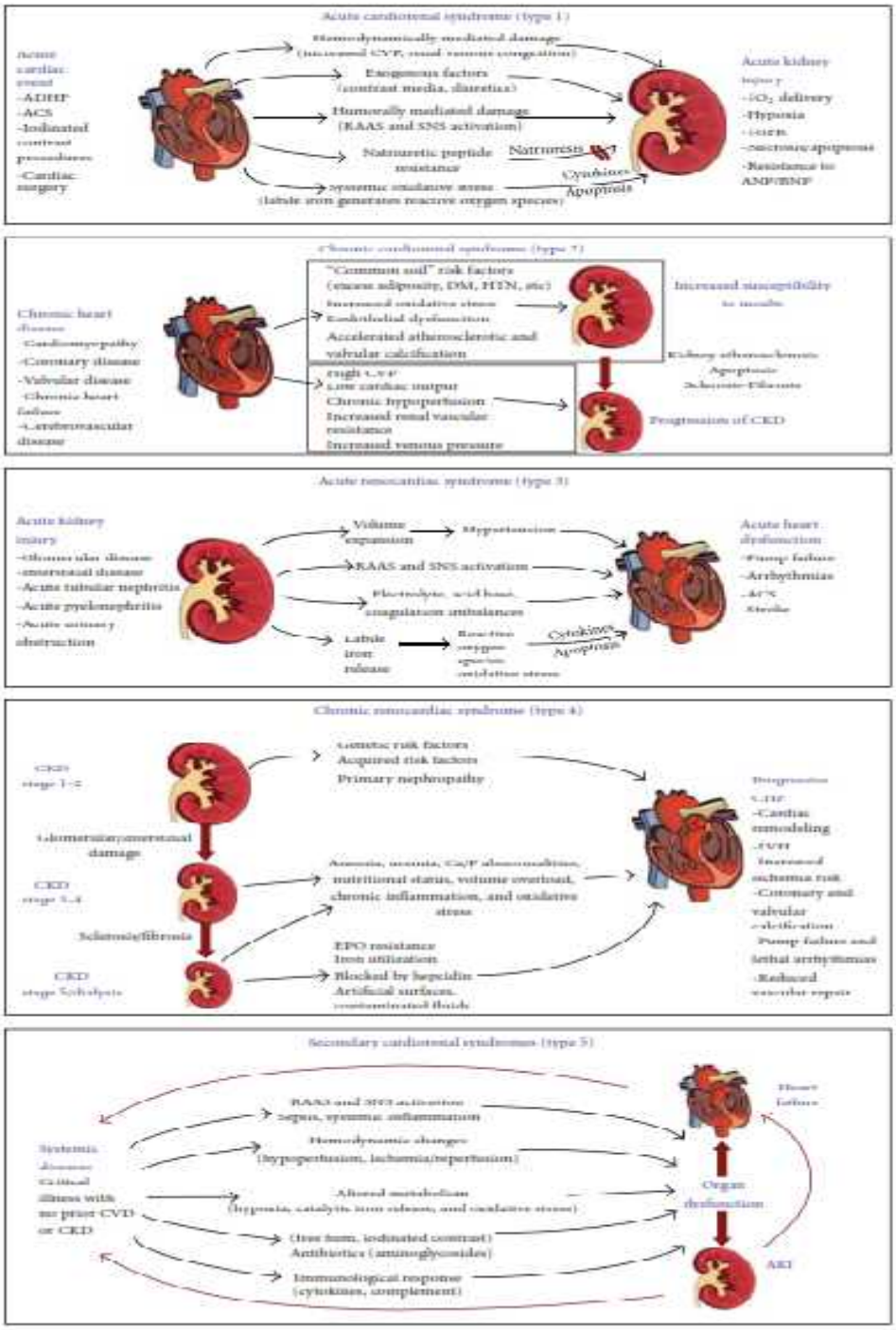

Figure 2. Definition and pathophysiology of different subtypes of CRS. From "Cardiorenal syndromes: pathophysiology to prevention," by P. McCullough, 2010, *Journal of Nephrology, 2011,* 2. Reprinted with permission.

The underlying etiology and pathophysiology of each type of CRS are similar to each other. While in Type 1 CRS acute injury to the heart was the primary source of pathophysiology, as demonstrated in Figure 2, Type 2 CRS entails patients who had kidney dysfunction or injury due to chronic repeated deterioration of heart function (Cruz et al., 2013). Type 3 CRS describes patients with primary acute kidney injury which leads to cardiac dysfunction (Bagshaw et al., 2013). In type 4 CRS, patients with initial chronic kidney disease eventually have cardiac injury and dysfunction (Tumlin et al., 2013). Finally, in type 5 CRS, different conditions in the body lead to renal and cardiac dysfunction (Mehta et al., 2013).

In most studies CRS 2 and 4 subtypes were challenging to discern due to the difficulty in determining if heart or kidneys was the primary source of dysfunction. Consequently, another proposal was to make a more general subtype known as CRS 2/4 (Bagshaw et al., 2008). There were also different researchers that grouped various cardiovascular diseases in different subtypes differently. In McCullough and Ahmad (2011), researchers chose to describe CRS type 2 and 3 as including acute coronary syndrome, stroke, and arrhythmias, instead of just CHF.

CRS epidemiology

According to multiple studies, researchers have determined that patients that have CHF, one of the most common comorbidities are renal dysfunction (Cruz, Gheorghiade, Palazuolli, Ronco, & Bagshaw, 2011). Additionally, McCullough (2002) indicated that six million Americans had a combination of chronic CVD and CKD, making the prevalence rate 2.1%. In individuals over the age of 65 years, an estimated 22% suffer

from the threat of CKD, diabetes, and CHF (Byham-Gray, Burrowes, & Chertow, 2010). According to the Acute Decompensated Heart Failure National Registry (ADHERE), as many as 21% with CHF have a serum creatinine > 2.0 mg/dl (Heywood, 2004). Additionally, 30% of the patients had renal failure. According to the Atherosclerosis Risk in Communities (ARIC) data studying 14,875 middle-aged subjects, those with estimated GFR at <60ml/min/1.73m2 had a crude CHF incidence of 17.7 per 1000 person-years compared to 5.7 per 1000 person-years in those with a GFR of ≥90ml/min/1.73m2 (Kottgen et al., 2007). Additionally, in those that developed heart failure or death experienced greater renal dysfunction than those that did not develop heart failure. According to the Evaluation Study of Congestive Heart Failure and Pulmonary Artery Catheterization Effectiveness (ESCAPE), 31.4% of the subjects who had CHF had a GFR of <60ml/min/1.73m2. When researchers later analyzed the subpopulation of 401 subjects who had a creatinine level recorded, they found that patients with a dynamic renal function, whether improving or worsening, had worse prognosis than those with stable renal function (Testani, McCauley, Kimmel, & Shannon, 2010). In another clinical trial known as the Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity (CHARM) trial, in 2,680 Class II-IV NYHA Heart Failure subjects, 36% had moderate to severe renal dysfunction (Hillege et al., 2006; Stojiljkovic & Behnia, 2007). After a follow-up of approximately 3 years, researchers found that in those with eGFR < 45 are 1.86 times more likely to suffer a heart failure hospitalization and cardiovascular death than those with CHF exclusively. The research suggests that adverse outcomes in different cohorts are exacerbated by CKD.

Researchers from another major clinical trial demonstrated the need to pay attention the renal component of CRS. The researchers from the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) clinical trial focused on how 2,737 patients who were given the aldosterone antagonist fared compared to placebo when they have an ejection fraction of 35% and Class 2 New York Heart Association Heart Failure (Cleland et al., 2011; Collier et al., 2013; Zannad et al., 2011). Patients were >55 years of age, 78% men, and followed for an average of 2 years. The results demonstrated a reduction of 37% in CVD hospitalization and death. Additionally out of patients with class 2 NYHA CHF, 33.4% had moderate to severe renal dysfunction. In another study, researchers from the Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) studied 2,013 Japanese CHF subjects and found that those with estimated GFR at <30 ml/min/1.73m2 were 2.5 times more likely to suffer all-cause mortality than those without CKD (Hamaguchi et al., 2009). Among individuals in the Digitalis Investigation Group (DIG) study, researchers found that 45% with CHF had concomitant kidney dysfunction. These studies demonstrate another study in which the cardiorenal component is critical to study as a syndrome (Hamaguchi et al., 2009).

Cardiovascular mortality and morbidity rates were as high as 45% in patients with advanced kidneys disease, creating more challenges to reducing mortality in this high risk population (Sharma, 2012, p. 6). When compared to an age matched non-CKD control, individuals with CHF have mortality rates which are 10- to 20-fold higher (Cruz& Bagshaw, 2010). Additionally, in 2010, 43.6% of individuals with CKD had CHF;

whereas, only 19.1% of individuals without CKD had CHF (United States Renal Data System, 2013). In several studies, the frequency of CKD has been as high as 63% in those individuals with CHF. However, while these conditions coexist, the challenge in these studies has been to determine which disease took place first (Cruz & Bagshaw, 2010). Together these conditions are known as CRS, which is defined as a syndrome that has the presence of renal dysfunction or the development of renal dysfunction in people with heart failure (Liu et al., 2012, p. 692). According to the National Kidney Foundation (2013), the direct cost of coexisting CKD and CHF was $19.4 billion in 2010. This accounted for 37% of all Medicare related CHF expenditures.

While morbidity, mortality rates, and expenditures are especially high in CRS, one of the main problems noted is that CRS is not fully understood in its entirety, and there are biochemical, hormonal, hemodynamics, and pathophysiological factors that can cause negative effects to the organs (Carubelli Metra et al., 2012, p. 272). The traditional pharmacological interventions that are available to treat CVD or CKD individually tend to cause serious negative interactions to the other organs when treatment is started and have detrimental effects on the other body systems as well (Carubelli et al., 2012). Public health professionals, researchers, and physicians look for ways to reduce the morbidity and mortality in these individuals; however there are no gold standard tests to evaluate sudden death in the population CRS (Sharma, 2012). Additionally, the direct associations between heart and kidney disease is not clear and not enough research is available to define proper treatment options and to reduce the morbidity and mortality in individuals with CRS (Asinger et al., 2011, p. 581; Herzog et al., 2011).

Pathophysiology of CRS

While researchers have clearly delineated the epidemiology of CKD and CVD, literature is sparse about the bidirectional relationship between the two conditions. Researchers have long established that the kidney plays a critical role in cardiovascular and that hypertension originating from dysfunction related to sodium epithelial channels can be resolved by renal transplantation (Weir, 2009). Furthermore, large clinical trials have demonstrated the link between renal and cardiac dysfunction. Weir et al. (2008) performed the Candesartan in Heart failure - Assessment of Mortality and Morbidity (CHARM)-added trial, which was a randomized control trial to demonstrate efficacy of reno-protective drugs like aldosterone receptor blockers in patients with congestive heart failure. However, since CRS has unclear etiology, some researchers look for common clues to the pathological response of the organs that are associated with CRS. Among these clues that have been observed include sympathetic nervous system upregulation, oxidative stress enhancement, increased activity of the renin-angiotensin system, and immune-mediated inflammation. These aforementioned pathological changes of oxidative stress and calcium overload many times causes observed abnormalities like left ventricular regional wall motion abnormalities as a result of hemodialysis (Zuidema & Dellsperger, 2012). Additionally, inflammatory markers are frequently studied because it is the body's response to protect the organs from infection; however, longstanding inflammation can cause problems with any organ in the body and eventually result in detrimental effects of the cardiovascular and renal systems in individuals with CRS (Colombo et al., 2012). According to a pilot study by Colombo et al. (2012), serum

samples from those individuals with Type 1 CRS demonstrated that pro-inflammatory cytokines like TNF-α, IL-6 and IL-18 were significantly higher than those individuals with acute heart failure and control. As is demonstrated in Figure 3, TNF-α, IL-1β, IL-6, and HS-CRP are all byproducts of CKD and CHF (Colombo et al., 2012). Additionally, researchers have found nitric oxide and COX-2 are other proinflammatory molecules released due to endothelial damage. These findings demonstrate that these individuals not only have immune dysregulation, but also more specifically dysregulation of apoptosis (cell death) (Virzi' et al., 2012).

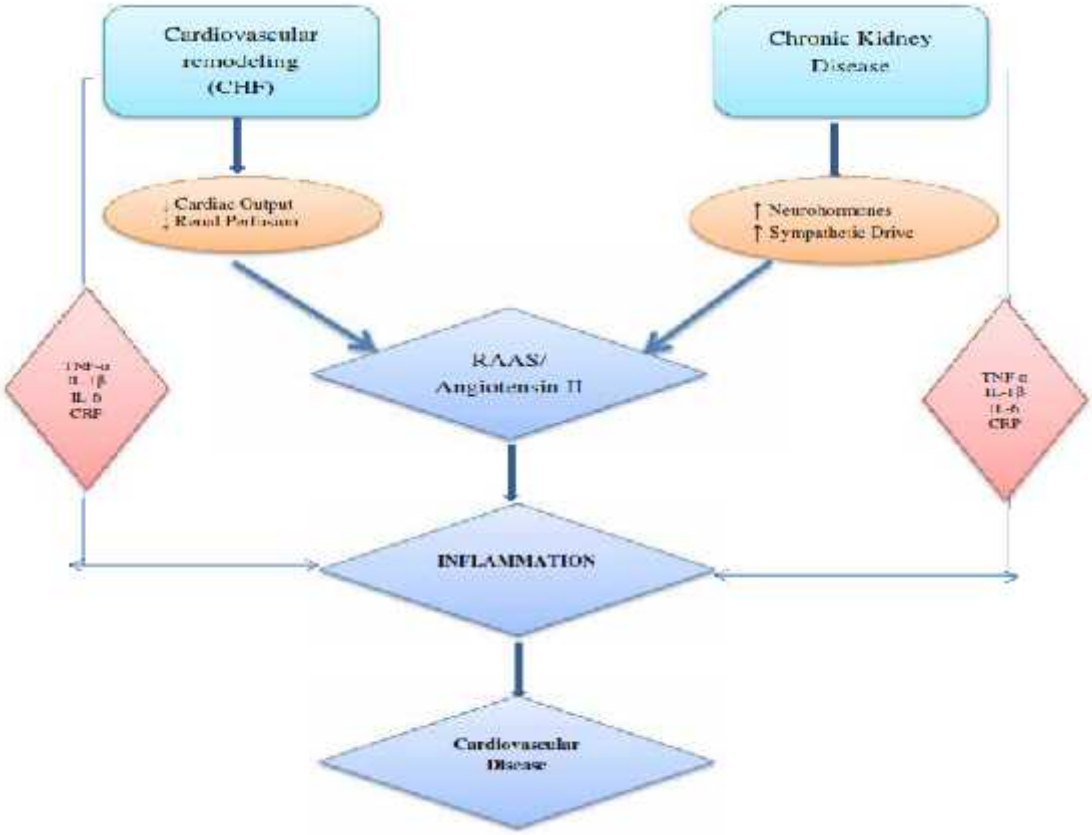

Figure 3. Potential Inflammatory Etiology of CRS. Adapted from "Inflammatory activation: Cardiac, renal, and cardio-renal interactions in patients with the cardiorenal syndrome," by P. C. Colombo, A. Ganda, J. Lin, D. Onat, A. Harxhi, J. E. Iyasere, and G. Atalay, 2012, *Heart Failure Reviews, 17(2),* 177-180.

Cardiorenal Anemia

Any discussion of cardiorenal syndrome is not complete without a discussion of anemia. This syndrome causes anemia by the fact that renal failure causes decrease in the release of erythropoietin. This hormone is responsible for erythropoiesis in the bone marrow. Additionally, anemia of chronic disease, which is induced by hepcidin production due to CRS, causes further deterioration of anemia (Nitta, 2011). The latter condition is primarily caused by macrophage retention of iron, decreased retention of iron

through the duodenum, and anemia from chronic inflammation. Chronic inflammation also causes a lack of responsiveness of erythropoietin. Due to the need to control anemia, according to the Anemia Working Group from the European Renal Best Practice (ERBP), hemoglobin levels should be maintained above 11-12 g/dL (Attansio, Ronco, Anker, Ponikowski, & Ander, 2010). However, in order to prevent overproliferation, they are of the opinion that hemoglobin levels should not exceed 13 g/dL in the population with CKD.

Prevention of CRS

Screening and prevention is a large component of CRS public health campaigns (McCullough et al., 2008). If the biomarkers are identified ahead of time, then the complications can be prevented in advance. Because CKD leads to accelerated atherosclerotic disease in the cerebral, coronary, and peripheral circulatory systems, management is more complex. According to the analysis from the Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort, researchers assessed the effect of screening the population of CRS biomarkers from a population size of 500,000 in northern Netherlands (Brouwers et al., 2013; Smink et al., 2012). After following a cohort for an average of 6.5 years, they found that in a targeted and specific population, like those subjects with albuminuria, screening is more effective than in performing it in a general population.

Because CRS is such a complex syndrome, the task of prevention can be quite challenging. As the pathophysiology becomes clearer, prevention strategies can be further elucidated. Authors in England, utilizing logistic regression, found that in order

to determine the likelihood of acute kidney injury, their CHF status is necessary (Forni et al., 2013). In contrast, when researchers utilized the traditional definition of WRF, subjects that demonstrated the aforementioned criteria showed only an additive effect in predicting unfavorable outcomes in the context of heart failure (Metra et al., 2012). However, in the retrospective analysis 949 patients in the Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure (OPTIME-CHF) study, individuals with >10 mg/dL change in BUN had a worse 60 day prognosis.

According to McCullough and colleagues (2010), an important area to consider for prevention is assessing the level of acute kidney disease the patient has using newer criteria. Based on the available data about what is acute renal failure, in 2002, a consensus conference formulated the Risk, Injury, and Failure; and Loss, and End-stage kidney disease (RIFLE) criteria. In terms of public health prevention programs, the period before the risk phase (R) when GFR has not decreased more than 25% and serum creatinine has not increased 150% to 200% is the time period to take prevention measures (Endre, 2008). Other researchers use different criteria known as Kidney Disease: Improving Global Outcomes (KDIGO) criteria and Acute Kidney Injury Network (AKIN) criteria in order to determine when the patient is experiencing AKI. In the context of CRS, researchers found that when the traditional WRF definition was used as compared to the newer definitions, the newer definitions had a benefit in determining unfavorable outcomes like death, dialysis, and readmission in those with Acute Decompensated Heart Failure (ADHF) (Roy et al., 2013). This research demonstrates the fact that new criteria need to be used to stratify those with cardiovascular disease.

Similarly another potential area of intervention and prevention is to identify individuals at high risk for cardiovascular disease and heart failure. While the original authors for the major risk scores like Framingham Risk Score and Reynolds Risk did not include diminished renal function as a major risk factor for cardiovascular disease, the authors of QRISK-2 included CKD as a risk factor of 10-year CVD (Chen et al., 2013; Collins and Altman, 2012; Ridker, Paynter, Rifai, Gaziano, & Cook, 2008; Santos, Vinagre, Silva, Gil, & Fonseca, 2010). In both of these risk-scoring systems inflammatory markers and kidney failure status is necessary.

Critique of Methods

Researchers have performed multiple studies which have assessed the connection of CVD and CKD in different ways. For instance Go et al. (2004) published findings in The New England Journal of Medicine assessing differential effective GFR outcomes among 1,120,295 adults who were part of the Kaiser Permanente Renal Registry, which according to modern classifications would be considered Type 4 CRS (Go et al., 2004). They were longitudinally studied, and using Multivariate Cox Proportional Hazards, researchers found that between 1996 to 2000, as the range of effective GFR worsened, the number of cardiovascular events increased (Go et al., 2004). While at a range of 45 to 59 ml/minute/1.73 m2 the Hazard Ratio (HR) of 1.4 (95% CI, 1.4-1.5), when GFR was less than 15 ml/minute/1.73 m2, the HR for CV events was 3.4 (95% CI, 3.1-3.8). The limitations of this study were that it only included insured people in northern California and did not take into account diabetes and hypertension as confounders (Go et al., 2004).

While the study is reliable due to sample size, the lack of generalizability jeopardizes the external validity.

While Go et al. (2004) assessed Type 4 CRS in a large population in northern California, Sato et al. (2013) studied if there was a difference in cardiovascular outcomes in CHF patients with CKD versus without CKD. A total of 505 patients with a mean age of 60 at Fukushima University in Japan with CHF were followed between the years of 2007 to 2010. Out of individuals with CHF who had concomitant CKD 34% had adverse cardiovascular events, whereas patients with CHF who did not have CKD only 14% had adverse cardiovascular events ($p < 0.001$). Additionally, through Multivariate Cox Methods, researchers found that pro-BNP was a significant biomarker in predicting adverse cardiovascular events in the group with both CKD and CHF. Similar to the study conducted by Go et al., this study also lacked generalizability due to its applicability to one community in Japan with good exercise tolerance.

Similar to Sato et al. (2013), another group of researchers studied cardiovascular outcomes in those CHF patients from Brazil who also had CKD (Galil, Pinheiro, Chaoubah, Costa, & Bastos, 2009). In this prospective cohort study, conducted between January to December of 2006, researchers found that among 83 CHF patients, the presence of CKD increased the chance cardiovascular outcomes by 3.6 times (CI 95% 1.04-12.67, $p = 0.04$) (Galil et al., 2009). While this study took many potential confounding variables like physical inactivity and cholesterol level into account, the small sample size might have compromised the power of the study. Additionally, due to

differences in diet and cultural differences, the results may not be generalizable to the United States.

Researchers from another major study assessed the relationship between renal function and cardiac function in a slightly different manner. Gutiérrez et al. (2013) published findings in the Journal of American Medical Association, from a prospective cohort study assessing differential associations of ACR (marker of renal vascular function) and coronary heart disease CHD stratified by race (blacks vs. whites) (Gutiérrez et al., 2013). The analysis was done on 28,207 individuals, 45 years and older, from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study between 2003 and 2007 and were followed up for 4.4 years (Gutiérrez et al., 2013). The most significant finding in this study was that in blacks, when medications and traditional CVD risk factors are controlled, the rate of incident CHD was depending ACR rate. The HR was 3.21 (95% CI, 2.02-5.09) when comparing ACR >300 mg/g vs <10 mg/g, while in whites the HR was not as significant (Gutiérrez et al., 2013). One weakness of this study was that due to many categories of ACR, the sample size was low in some categories, potentially affecting power. Additionally, because other races were excluded, the generalizability of the study could be called into question.

The continuous NHANES dataset was used previously in many analyses to assess different variables associated with cardiovascular disease (Bansal, Vittinghoff, Plantinga, & Hsu, 2012). In this study, Bansal et al. (2012) used a cross-sectional analysis on 34,326 subjects between the years of 1999-2006, in order to study if CKD modified the association between BMI and CVD risk factors. Out of the CVD risk factors tested as

outcome variables, one of the outcome variables tested was C-reactive protein (Bansal et al., 2012). The conclusion was that there was no association between BMI and risk factors, when race, sex, and age were used as covariates. Regardless of the presence or absence of CKD, an increase in BMI was associated with an increase in CRP in the same way and had no statistically significant difference ($p=0.095$). This study had robust power due to the large sample size (Bansal et al., 2012). Due to the use of the NHANES dataset, the weaknesses were the lack of knowledge of other comorbid conditions and use of self-reported data. Additionally, the absence of stroke and arrhythmia as cardiovascular disease led to possible inaccurate results.

Knowledge Gap

As reflected in the previous section, many researchers have studied the impact of CKD of CVD (Bansal et al., 2012; Go et al., 2004; Gutiérrez et al., 2013; Sato et al., 2013). These studies were only representative of small communities and did not reflect a larger population (Go et al., 2004; Sato et al., 2013). In other studies, the definition of CVD was too narrow, making the results inaccurate (Bansal et al., 2012). In recent years, researchers have increased their interest in the role of inflammatory biomarkers (Bansal et al., 2012; Lekawanvijit et al., 2012). In the context of obesity, diabetes, and metabolic syndrome, out of all the biomarker, hs-CRP has been studied repeatedly. Researchers have demonstrated the need to screen hs-CRP in especially high risk individuals with CRS in smaller populations in different countries like Bavaria, Korea, Brazil, and Spain (Jalal, Chonchol, Etgen, & Sander, 2012; Park & Kim, 2003; Soriano, González, Martín-Malo, Rodríguez, & Aljama, 2007; Xue et al., 2010).

Due to the recent establishment of the definition of CRS, there has been very little research done on the relationship of biomarkers in this syndrome in particular in a nationally representative population (Xue et al., 2010). While Soriano et al. (2007) found in CKD patients with high hs-CRP increased cardiovascular events in a small community in Spain, no researcher has studied how different biomarkers interact with known CKD and CVD risk factors in a nationally representative population (Ronco, Cicoira, & McCullough, 2012; Tumlin et al., 2013). Very little research has been done in studying how to prevent CRS, due to the complexity of the syndrome (Lekawanvijit et al., 2012). Understanding the epidemiological association in specific cardiovascular diseases and CKD can lead to identifying critical biomarkers that could signal clinically aberrant manifestations before they occur (Xue et al., 2010). Additionally, literature is scant on how upstream demographic factors potentially differentially interact with inflammatory markers in associations with CRS in a nationally representative population (Gutiérrez et al., 2013). By investigating and addressing the importance of inflammation as potential biomarkers, public health practitioners and healthcare advocates and providers can effectively empower patients to improve their own health and educate patients about how to control inflammation and slow or prevent disease progression.

National Health and Nutritional Examination Survey

The National Health and Nutritional Examination Survey (NHANES) was started in 1971 with the intent to quantitatively and qualitatively measuring the nutritional and health status of children and adults (McDowell, Engel, Massey, & Maurer, 1981). Additionally, the intent was to assess the prevalence of major diseases and understand

risk factors. This annual survey is conducted in the United States and includes socioeconomic, demographic, exposure-related, and dietary information. Researchers and health practitioners ask subjects to provide blood and urine samples with the intent of measuring exposure and nutritional exposures.

Summary and Conclusion

This chapter entailed a thorough literature review on the inflammatory etiology of CVD. Additionally, there was discussion on the risk factors of CVD and the definition of CKD. Furthermore, CRS was specifically defined, and the epidemiology was delineated. Finally, there was a thorough discussion on the potential etiology and different subtypes of CRS. While multiple etiologies have been proposed on the role of inflammation on CRS, how the specific inflammatory markers are connected to CRS is not well known. Additionally, the current research will not only fill the gap on the impact of inflammatory markers on CRS, but also delineate how demographic factors play a role in this relationship between inflammatory markers and CRS. Chapter 3 will include the research method that was utilized for this study. The design and approach of the research, population sample, data collection, and data analysis method was included.

Chapter 3: Research Method

Introduction

CRS, a growing public health burden, is associated with increased mortality, growing complications, and increased cost of care (Lekawanvijit, Kompa, Wang, Kelly, & Krum, 2012). Chronic inflammation is a multifactorial effect that has become epidemic throughout the world as a result of chronic diseases like diabetes, hypertension, hyperlipidemia, and chronic kidney disease. A significant increase in chronic disease prevalence among all populations has become a primary public health issue mainly because risk factors like diabetes and obesity are on the rise.

The purpose of this study was to evaluate whether specific inflammatory markers make CRS more likely in the general population. According to literature reviews, several factors such as gender, race/ethnicity, age, education, socioeconomic status, obesity, hyperlipidemia, and diagnosis of diabetes mellitus have been previously associated with CVD and CKD individually (Graves, 2012; Hui et al., 2013; Wong et al., 2012). The risk factors that were utilized in the data analyses were used because of the potential confounding effect and have been delineated in greater detail in the data analysis section.

The current chapter entails critical information about the methodical approach to selecting the proper research question, selection criteria of the research design and approach that was utilized, setting of the study and the specific sample method, data collection method, and various statistical methods that was employed to test hypotheses of the association between inflammatory markers and CRS. Additionally, power considerations for the appropriate sample size selection, ethical and legal considerations,

and the specific biases which threaten empirical, content, and construct validity are delineated in detail.

Research Design

Research Approach

In a nationally representative population, the goal of the present study is to conclusively determine whether inflammation is a risk factor for CRS. In this study, the quantitative approach was selected to study this association because this method is the most appropriate to understand the relationship between numerous variables. According to Creswell (2009), this approach is better for understanding new theories, determining specific proportions, and testing multiple hypotheses. Researchers who employ quantitative methodology for analysis utilize a methodical deductive approach. Alternatively, the study is driven by measurement represented by numbers in order to quantify the outcome (Trochim & Donelly, 2008). From the general population in the United States, NHANES is one such survey where health and nutritional data have been collected for over four decades and the survey and examination techniques have evolved and made to increase the public policymakers. This study will explore the association between elevated serum inflammatory levels and CRS both independently and additively with known CVD risk variables like obesity, age, gender, diabetes status, hyperlipidemia, and smoking status.

Research Rationale

The nature of this study will have a quantitative focus. This secondary data analysis on cross-sectional data is consistent with developing a thorough understanding in

a multi-ethnic population (non-Hispanic whites, non-Hispanic blacks, and Mexicans) on how inflammatory biomarkers can signal the subsequent development of renal failure, with the development of cardiovascular disease. Inflammatory markers and their association with cardiovascular and renal disease was taken into account, while controlling for known cardiovascular risk factors like obesity, age, gender, diabetes status, and smoking status (Levitan et al., 2009). The inflammatory markers and demographic data were included in the respective datasets. Survival analysis was employed to assess the differential development of cardiovascular disease between demographic groups and different inflammatory status in individuals with chronic kidney disease.

The main goal of this study is to determine whether inflammatory markers play a significant role in CRS. In order to study this relationship, quantitative methodology was utilized due to the robustness in examining numerous variables, determining proportions, formulating new theories (Creswell, 2009). In order to test the hypothesis of this association, quantitative methodology can be used through deductive reasoning. This distinct methodology is utilized when determining the theory is the main goal and is derived from quantitative measurements that made from outcome measurements (Frankfort-Nachmias & Nachmias, 2008; Trochim & Donelly, 2008).

Out of other alternatives, the NHANES survey was selected due to multiple reasons. First of all, all of the different information like detailed demographic information, inflammatory markers, and past medical history information has been systematically collected on a nationally representative population. Additionally, between

the years of 1999-2010, researchers have collected enough data in order to study CRS, providing a large sample size in a population who were 20 years or older. Because CRS is an uncommon syndrome, a large range of years are necessary to capture an adequate number to have adequate power for a study.

The main theory that is at the crux of the study is the biomedical model in which researchers purport that CRS is a consequence of biological aberrations in the body. More specifically, biomarkers can be used as an early marker for identifying the eventual consequence of CRS (Mayeaux, 2004). Similar to diabetes, increased inflammatory markers have been linked to cardiovascular disease and renal disease individually. Consequently, it is highly likely that increased inflammatory markers will play a role in both of these diseases simultaneously. Exploring the association of inflammatory markers and CRS was tested using the presence of biomarkers like C-reactive protein, hcy, ferritin-transferrin ratio, and fibrinogen.

The cross-sectional design was appropriate for this study because the specific prevalence of the disease of interest and outcomes of health can be ascertained from surveys and clinical records in the NHANES. Since CRS is a newer classification, this dataset allows for a unique perspective into the syndrome which is not studied routinely. Additionally, the same design can be replicated or the study can further extended to a longitudinal study in order gather and obtain more information on this specific problem (Creswell, 2009). Rather than incidence, these findings will provide information on prevalence (Levin, 2006). A causal relationship is difficult to ascertain because it is conducted in a single moment in time, making this a major limitation (Singleton & Straits,

2005). Even though there are limitations, this study design is the most appropriate because it is the first study to fill a gap on the role of inflammatory markers as a risk factor for CRS in a multiethnic nationally representative population.

Alternative study designs were inappropriate due to different reasons. A case-control study was not practical in studying CRS in the context of inflammation because the time to develop the constellation of symptoms in the syndrome may be longer than others. Additionally creating a prospective control, retrospective control, or experimental study would have been time consuming, expensive and unusually complex (Singleton & Straight, 2005). Only when specific associations are established are these designs applied. Therefore, because this study is the first study to explore whether inflammatory markers are a risk factor for CRS, cross-sectional design and the quantitative methodology were utilized to analyze relationships derived from the most recently acquired data (1999-2010) collected by NHANES.

While C-reactive protein, hcy, ferritin, and fibrinogen have been studied in the past, there is a gap in the literature in understanding how they interact in the context of CRS. Furthermore, how sociodemographic indicators modify the relationship between inflammatory markers and CRS remains unknown. The joint effect of cardiovascular risk factors and inflammation on CRS in a multiethnic population has not been assessed in any other study.

Study Sample and Population Setting

The NHANES dataset that was previously collected by the NCHS and is available for public use was utilized for this study. Data from individuals who reside

with the general United States population was utilized in this retrospective secondary data analysis for the quantitative research study. Civilian, non-institutionalized adults who reside within the continental United States was included as part of this study. There were 62,160 individuals who participated in the questionnaire component only and 59,367 NHANES who participated in the questionnaire and examination component in the survey years 1999-2010. Of the adult men and women, a net total of 51,130 individuals who took part in the NHANES survey years 1999-2010 aged 20 years and older, were incorporated into the analysis. The six 2-year cycles of the continuous was used for the purposes of this analysis. Age was used as a primary variable for inclusion in the secondary analysis because this study is only pertaining to inflammatory markers associated with chronic diseases affecting adults. According to previous research, at least 2.1% has CRS out of the general population (Cruz et al., 2011; McCullough, 2002). Questions specifically asked by the researchers of the NHANES survey, in addition to data collected from laboratory analysis and medical examination were used to study the identified population. The aspects of the NHANES datasets that was included are: questions related to height and weight; history of diabetes; history of chronic kidney disease and heart disease; history of smoking; and serum collected for analysis of hs-CRP, hcy, ferritin-transferrin ratio, and fibrinogen (CDC, 2011).

Power Analysis

The NHANES data sets include 51,130 responders who are aged 20 years or older from the aforementioned dataset. Results from previous analyses demonstrate that 2% of the US population has CHF with 60% who have concomitant CKD (Adams et al., 2005;

Ezekowitz et al., 2004; Heywood et al., 2007; Hillege et al., 2007; Smith et al., 2006). As determined by McCullough (2002), using the 2.1% as an estimate for the prevalence of cardiorenal syndrome, the calculated total number with CRS out of the NHANES datasets (51,130 subjects) was determined to be 1074 subjects. In order to determine whether the total sample of 614 participants was adequate for this analysis, calculations were made using he G*power utility **(**Buchner, Erdfelder, Faul, & Lang, 2013).

The main analysis for this study was a logistic regression. For a logistic regression analysis, sample sizes of 568 will yield a power of 80% with $\alpha = 0.05$ and a minimum detectable odds ratio of 1.3 with inflammatory markers as the predictor variable and CKD, CVD or CRS as the outcome variables when testing the different hypotheses **(**Buchner, Erdfelder, Faul, & Lang, 2013; Muncer, Taylor, & Craigie, 2002). The minimum detectable odds ratio of 1.3 was used because based on the odds ratio in previous research (Skinner, Steiner, Henderson, & Perrin, 2010). Additionally, according to Vittinghoff and McCullough (2006), each predictor variable conservatively must have ten outcome events per predictive variable in order to prevent overfitting, under special circumstances less than ten is permissible. In light of these calculations in support of the power analyses, there was confidence that the sample size of 51,130 participants with anticipated 614 participants with CRS was adequate to perform the analyses in the present study.

Instrumentation and Materials

The secondary analysis was performed retrospectively using archival data in the NHANES database, which is readily accessible from the internet (CDC, 2011). The

NHANES survey is derived from a cluster sample design with a complex, stratified, and multistage survey. In the process of collecting data for NHANES, the first part is sample selection. The sample selection is performed methodically in stages by first selecting Primary Sampling Units (PSUs) (CDC, 2011). These are either sets of counties that are continuous with each other or larger counties. Next, a cluster of households or segments which are a component of a group of blocks or PSUs are selected. After this, households that compose the segments are selected. Finally, specific participants within households are selected. Those identified as fitting the appropriate inclusion criteria were included in the NHANES sample by sending them a letter in advance, informing them that an interviewer from NHANES was going to visit their home. Once the home visit took place, the interviewer proceeded to recruit who are eligible to participate in NHANES. They assured the individuals confidentiality of the data collected from the survey, informed the respondents of their rights, and requested them to sign the form for Informed Consent. A computer-assisted personal interview (CAPI) system using the Blaise computer program was used (CDC, 2011). After completion of the household interview, persons that were interviewed were requested to complete a component about health information. This was only performed on individuals who signed an additional consent form agreeing to participate in the health examination part.

Validity and Reliability of the Instrument

Validity

In order to ensure validity of each measurement and instrument used throughout the study, special precautions needed to be taken during the collection and analysis

component of the study. Because the NHANES has a long history and has been collected since the 1960s (earlier version was known as the National Health Examination Survey), the validity of the survey has been repeatedly tested throughout the years on over 130,000 study participants (Birkner, 1965; Khrisanopulo, 1964; McDowell, Engel, Massey, & Maurer, 1981). Specific errors that had emerged have been controlled for by making changes in the creation, development, implementation, and administration of the survey. In the NHANES data, the types of validity that was explicated are as follows: empirical validity, content validity, and construct validity (Frankfort-Nachmias & Nachmias, 2008).

Empirical validity. Researchers from National Center for Health Statistics (NCHS) go through different measures to ensure that the NHANES is empirically valid (CDC, 2011). In terms of the NHANES survey, researchers had to ensure that the questions measured the outcomes needed to be measured. For instance, in measuring hs-CRP, they had to make sure that the labs that were using had a consistent protocol in place in order to measure this critical biomarker. This is closely interrelated with predictive validity where the measures can be helped to ascertain associations expected to be obtained. In numerous secondary analysis studies, SAS and SPSS have been utilized in order to analyze NHANES data. In this study, these two statistical packages was utilized to follow suit with what packages was used in order to confirm and ensure empirical validity to the study proposed here (CDC, 2011; Frankfort-Nachmias & Nachmias, 2008; Petrou, Morrell, & Spiby, 2009).

Content validity. In this study, the content validity of the NHANES survey is ensured by various means, and also specific measures are necessary in order to minimize

bias. Through stratification and cluster design of the survey, content validity can be ensured. Additionally, the NHANES survey uses objective measurements like examination and laboratory data along with questionnaire results. In summary, the NHANES survey ensures not only face validity, which is a subjective evaluation of appropriateness, but also sampling validity. The following descriptions of selection bias and information bias demonstrate how these are minimized in order to ensure content validity (CDC, 2011; Frankfort-Nachmias & Nachmias, 2008).

Selection bias. When selecting individuals that are adequate for a specific research question, there is potential bias in determining and selecting individuals who are with or without disease. Researchers from the Centers for Disease Control and Prevention used a complex and systematic approach in order to ensure randomness and minimize selection bias. The steps they took were as follows: 1) they selected Primary sampling units (PSUs); 2) they divided the PSUs into neighborhoods; 3) they selected random households from the neighborhoods; and 4) Depending on the gender and ethnicity of each household, 1.6 individuals per household were selected (CDC, 2011). This systematic approach ensured the representativeness of the sample throughout the general population and ensures the presence of external validity. Historically, in previous versions of NHANES, high nonresponse rates have led to the potential for bias. For instance, according to Forthofer (1983), NHANES II had a nonresponse rate of 27% during the examination phase, in the first years. In order to address these issues, some techniques that were used included better follow-up methods than previously used. They started to market and advertise the success stories, in especially those communities that

the survey took place in. Additionally, they incorporated an introductory letter, and the interviewer began by verbally expressing the significance of NHANES during each household visit. Other techniques that were used were providing incentives to encourage participation. For instance, participants were provided with a free medical education, free transportation, and guaranteeing confidentiality. In order to further adjust for nonresponse and adjust for unequal probability of selection, NHANES investigators provide a separate sample weight variable. Consequently current non-response rates have gone down to 20% and are much lower than the 55% nonresponse rates seen in Behavioral Risk Factor Surveillance System (BRFSS), another national self-reported questionnaire (Hu, 2008, p. 17).

Information bias. Information bias is an error that is systematically made during the process of making measurements of the participant study. There are two primary types of misclassification—differential or non-differential misclassification. In this study, the primary source of information bias is the recall bias, which is part of a differential misclassification. In this type of bias, respondents may not be able to accurately recall information accurately because they are based on certain previous experiences or diagnoses that were given to them in the past. Some of the data from the NHANES data may contain self-reporting bias because the interview component depends on accurately recalled information by the participants of events from the past. In general, subjects that felt their information would be negatively perceived underreported the frequency of the incident (Klesges et al., 2004). This type of information bias is a combination recall bias and social desirability bias. In another recent study, Canadian

researchers reported that out of 4,615 subjects, men and women overestimated their height and underestimated their weight, resulting in 15% decreased rate of obesity (Elgar & Stewart, 2008). Other researchers found that among NHANES respondents between different years, with similar discrepancies in height and weight measurements resulted in an underestimation of the corresponding BMI (Connor, Gorber, & Tremblay, 2009). In order to minimize this information bias, the variables that was primarily studied was taken from anthropometric measurements and laboratory results rather than self-reported data. C-reactive protein and obesity data are all collected from direct measurements. In order to control for self-report bias, Hunt et al. (2003) proposed that direct and systematically objective measurements which are unbiased need to be utilized whenever available.

Construct validity. Researchers at the NHANES ensure the construct validity by employing different strategies. With construct validity it is important to ensure that the measurement technique being used yields the same result as other measurement techniques. For instance if there are two health care taking blood pressures of different people, the measurement technique employed by both health care worker must be consistent in order to yield meaningful findings. Because the NHANES survey has been collected for many years on the U.S. population, the measurement techniques are time tested methods. Inferentially, construct validity can be demonstrated by yielding similar from previous studies in regards to HS-CRP level, cardiovascular disease, and CKD (Frankfort-Nachmias & Nachmias, 2008).

Reliability

By controlling for errors in measurement, reliability must be preserved in a given study. In this study, multiple steps were taken to make sure that there is reliability in the instrument. On the data collection level, this took place by, first, checking the data transcripts and matching them with the respective windows on the CDC website. Once the data had been checked, the ordinal, nominal, and ratio data variables were accurately coded and they must be given numbers, ranked accurately, and dummy coded properly with the fixed values. Next, data collection, data analysis, and assignment were discussed in regular meetings with staff members to ensure uniformity and reliability. Finally, the data was cross-checked with other colleagues to compare and cross-reference the accuracy of the analysis of the data. As mentioned previously, a time tested instrument added to the reliability and credibility of the NHANES survey (Frankfort-Nachmias & Nachmias, 2008).

Study Variables

The research study variables were selected based on current research interest, literature review, and the availability if the collected data in the NHANES survey. Weight, height, blood pressure, and blood tests for inflammatory markers were performed on the study population at the mobile examination center by NCHS. Other variables, such as diabetes, age, gender, education level, socioeconomic status, smoking status were obtained from interviews with the study population. The specific details about each individual survey procedure are thoroughly described in detail in the accompanying documentations which were provided by the NCHS (CDC, 2011).

Dependent Variables

Chronic Kidney Disease. A trichotomous CKD variable was created using the KDOQI (Kidney Disease Outcomes Quality Initiative) study classification for stages of CKD (Tan et al., 2011). Before CKD can be categorized, a urine albumin-to-creatinine ratio (ACR) was calculated. Urine creatinine and albumin concentration were ascertained from random spot urine samples using sterile containers and a clean-catch technique. As previous researchers have indicated, the categories for the ACR >30 can be defined as albuminuria and a reflection of early CKD (Inker, Coresh, Levey, Tonelli, & Muntner, 2011).

For demographic purposes, individuals in stages 3–5 (eGFR of 0–59 mL/min/1.73 m^2) was classified as having late CKD (coded 2); individuals in the stages 1 and 2 (eGFR of 60–89 mL/min/1.73 m2) or eGFR of greater than 90 mL/min/1.73 m2 with ACR>30) was classified as having early CKD (coded 1) (CDC, 2011; Whaley et al., 2008). All other individuals were classified as having normal kidney function (coded 0).

Cardiovascular Disease. In this evaluation, cardiovascular disease can be defined as coronary heart disease, congestive heart failure, angina, cerebrovascular accident, and heart attack (coded as 1). The presence or absence of all of these diseases was based on self-reported data (CDC, 2011). All reports were based on if the study participant was or was not informed by a physician if s/he has had one or more of any of the aforementioned conditions. The rest was coded as 0.

Cardiorenal Syndrome. Cardiovascular syndrome can be defined as all individuals with eGFR less than 60 mL/min/1.73 m^2 (1.0 mL/s/1.73 m^2) or ACR of 30

mg/g or greater and having self-reported CVD. More specifically, any individual with coronary heart disease, congestive heart failure, angina, cerebrovascular accident, and heart attack and 60 mL/min/1.73 m^2 or ACR of 30 mg/g (CDC, 2011).

Independent Variables

In studying CRS, because the variables are bidirectional, cardiovascular disease and CKD will used as dependent or independent variables depending on the question being answered. The main independent variable for this study was inflammatory markers hcy, ferritin-to- transferrin ratio, fibrinogen, and hs-CRP.

Homocysteine. Total plasma hcy levels were determined using the fully automated fluorescence polarization immunoassay (Abbott Laboratories). As previous researchers have indicated, the categories for the hcy are 2.0-6.9 μmol/L, 7.0-8.7 μmol/L, 8.8-11.0 μmol/L and > 11.0 μmol/L. According to researchers, ≥ 8.5 μmol/L (≥50^{th} percentile coded 1) is considered a cardiovascular risk factor, while the rest (coded 0) is not (Foley, Wang, & Collins, 2005; Mangoni & Woodman, 2011; Veeranna et al., 2011). Hcy data collection was cycled out in 2007 (CDC, 2011).

Ferritin-to-transferrin saturation-ratio (F/T). A ferritin based inflammatory marker controlling for status of iron is the purpose of the F/T ratio. Because there are no specific clinical cutoffs that actually are present for this measure, in previous studies the defined F/T saturation was considered high when the value was greater than the weighted 95th percentile in the final analytic sample (Skinner, Steiner, Henderson, & Perrin, 2010). Following suit from a previous study, for categorical analysis, F/T was considered

elevated if it is ≥ 11.67 (≥50th percentile coded 1), the rest (coded 0) of the study population was considered not (Skinner et al., 2010).

C-Reactive Protein. Hs-CRP was quantified by using latex-enhanced nephelometry, and a high-sensitivity assay was performed on a Behring (Deerfield, IL) nephelometer. The lower limit of detection for this test was 0.2 mg/L. For continuous analyses, all values of 0.2 mg/L were coded as equal to 0.1. Research in adults has indicated increased risk of cardiovascular disease beginning at values of 1.0 mg/L, so this value was used as a cut point for analysis (Ridker, 2003; Ridker et al., 2005). Values of hs-CRP >0.51 (greater than 75th percentile coded as 1) was considered as cardiovascular risk, and hs-CRP < 0.09 (less than 25th percentile coded as 0) was not.

Fibrinogen. Fibrinogen concentration in plasma was determined using the Clauss clotting method. This test method involves measuring the rate of fibrinogen to fibrin conversion in a diluted sample under the influence of excess thrombin. Since under these conditions the fibrinogen content is rate limiting, the clotting time can be used as a measure of the concentration of the fibrinogen and in fact, the clotting time is inversely proportional to the level of fibrinogen in the plasma. A fibrinogen level of greater than or equal to 378 is considered increased risk of CVD, CKD, or CRS because this was greater than the 50th percentile (Dhangana, Murphy, Pencina, & Zafar, 2011).

Covariates

Age. The age of each subject was ascertained according to the provided age or date of birth provided by the respective subject. In this study, age categories was dummy coded as follows; 20-34 (coded 1), 35-49 (coded 2), 50-64 (coded 3), and 65 and older

(coded 4). These categories were used for the descriptive statistics section providing the age distribution for the study. In all other statistical analyses, age was used as a continuous variable to the study.

Gender. The respondents of the NHANES study were asked whether their gender was either male or female. In the study, gender categories were dummy coded as either "Male" (coded 1) or "Female" (coded 2).

Diabetes. Diabetes was ascertained from the NHANES questionnaire about diabetes. All respondents over the age of 20 were asked "Other than during pregnancy, have you ever been told by a doctor or other health professional that you have diabetes or sugar diabetes?". For the purposes of this study, the participants who answered "borderline" or "yes" was considered as diabetic (dummy coded as 1). However, those participants who answered "no" were considered as non-diabetic (dummy coded as 2). Respondent answers such as "don't know" "refuse" or "not sure" was marked accordingly as missing data and will not be included in data analyses.

Hyperlipidemia. Each respondent was asked about their cholesterol status. Previous researchers found that there was more data available on total cholesterol than LDL, so as per Adult Treatment Panel III guidelines, total cholesterol was used as the marker for hyperlipidemia (Chatterji, Joo, & Lahiri, 2011). Dyslipidemia or hyperlipidemia (coded as 1) was ascertained and determined to be present with use of cholesterol lowering medications, self-reported diagnosis, or a total serum cholesterol of > than 240 mg/dl. In subjects with diabetes, this number was > than 200 mg/dl and also coded as 1. The rest of the subjects were coded as 0.

Obesity. A well-recognized unit of measurement of obesity in the scientific community is the BMI. The unit is categorized according to the varying grades of health risk (WHO, 2006). In this study, BMI was arranged into four different categories. The first category is participants with BMI < 25, which is considered normal weight (dummy coded as 1); participants with a BMI = 25-29 was considered overweight (dummy coded as 2); participants with a BMI 30-39.9 was considered as obese (dummy coded as 3); and participants with a BMI >40 was considered severely obese (dummy coded as 4). BMI categorization is controversial because distinguishing between weight from fat versus weight from muscle (Faeh, Marques-Vida, Chiolero, & Bopp, 2008; WHO, 2006).

Hypertension. Hypertension (coded as 1) was defined as average systolic/diastolic blood pressure ≥140/90 mm Hg or self-reported antihypertensive medication use. The rest was considered as non-hypertensive (coded 0).

Smoking Status. A two-variable indicator of current smoking status was created with "nonsmoker" (coded 0) and "smoker" (coded 1). The subject was considered a "smoker" if s/he reports "yes" to the question, "Have you smoked at least 100 cigarettes in your entire life?" and did not answer "not at all" to the question, "Do you now smoke cigarettes…" in the NHANES 1988-1994 and 1999–2010 (CDC, 2011). Any answers by the participants such as "don't know", "refuse", or "not sure" was recorded as missing data and will not be included in the data analyses

Education level. The question "What is the highest grade or level of school you have completed or the highest degree you have received?" was the basis by which education level of the respondents was determined. In order to be consistent with

associated literature, eight categories were narrowed down to three categories for education level. The three categories that were dummy coded are as follows: “Less than high school” (coded 1), “High school graduate or GED” (coded 2) and “Higher” (coded 3). Other answers like “refused” or “don’t know” was marked as missing data (CDC, 2011).

Race/ethnicity. In order to ascertain the race/ethnicity of the subject, the interviewer asked “What race do you consider yourself to be?” Within the NHANES questionnaire, respondents were allowed to select one out of five categories. The different categories were dummy coded as; Non-Hispanic White (coded 1), Mexican American (coded 2), Non-Hispanic Black (coded 3) and Other (coded 4) (CDC, 2011). Any other answers by the participant such as “refused” or “don’t know” was marked as missing data (CDC, 2011).

Socioeconomic status. The socioeconomic status was based on poverty income ratio (PIR) included in the questionnaire. PIR, established according to the US Census Bureau (2012), was established based on the estimate of the per annum income of a family income per year and the size of each family. Groups of individuals were classified as families if they were related through marriage, adoption, or by birth. Other scenarios that were considered as family were parents or siblings or unmarried partners who had a biological or adopted child that was in common were considered as a family unit as well (CDC, 2011). In this study, the socioeconomic status of the family was categorized as follows; PIR <2 ($<$ 200% federal poverty rate) was labeled as poor (coded 1) and PIR ≥ 2 was considered as not poor (coded 2). Answers by the participants such as “don’t know”,

"refuse", or "not sure" were labeled as missing data and were not used in the data analyses.

Data Categorization and Analysis

Table 1 demonstrates the nature of all the variables that were utilized in this study. The reason the inflammatory markers was continuous and categorical is because in the context of multivariate analysis, they was categorized into levels that have been previously shown to be associated with CVD. This was discussed more thoroughly in the study variables section.

Table 1.

Different Variables and the Corresponding Types of Variables

Section/Data Set Title	Variables	Variable Type
Demographics (Demo)	Gender	Categorical
	Race/Ethnicity	Categorical
	Age Group	Categorical
	Poverty Income Ratio	Categorical
	Education Level	Categorical
Medical Conditions	Cardiovascular Disease (ACS, stroke, arrhythmias, congestive heart failure, and coronary heart disease (CHD))	Categorical
Medical Conditions	Chronic Kidney Disease	Categorical
Medical Conditions	CRS (ACS, stroke, arrhythmias, CHD, and CKD)	Categorical
Medical Conditions	Diabetes	Categorical
Medical Conditions	Hyperlipidemia	Categorical
Medical Conditions	Obesity	Categorical
Medical Conditions	Hypertension	Categorical
Body Measures	Body Mass Index (kg/m^2)	Continuous
C-Reactive Protein (hs-CRP)	C-reactive protein (mg/dL)	Continuous and Categorical
Hcy	Hcy (µmol/L)	Continuous and Categorical
Ferritin/Transferrin Ratio	Ratio controlling for other sources of iron increase	Continuous and Categorical
Fibrinogen	Fibrinogen (mg/dL)	Continuous and Categorical

.

All statistical analyses were performed using SAS 9.3 and SPSS 21.0 software (SAS, 2010). The main goal in this particular study was to answer the research question whether inflammatory markers are risk factors of CRS, and if sociodemographic factors modify this effect. Because the direction of the relationship is unknown, being that this

is the first study addressing this relationship, the two-tailed t-test if the distribution is normal, or Mann-Whitney U statistical analysis was utilized if the distribution was nonparametric (Zhao, Rhardja, & Qu, 2008). Therefore, based on what is available in the literature, the type of data available in the dataset, and NHANES recommendations, the alpha was set to 0.05 (CDC, 2011; Sun, Pan, & Wang, 2011). In other words, the probability of the association to happen by chance is 5%. Univariate analysis was performed to assess the gender, age, and racial distribution of the selected sample. Bivariate analyses was performed to evaluate the association between independent variables (age, race/ethnicity, PIR, etc.) of the study participants and to evaluate the relationship to dependent variables (CRS). More specifically body mass index (normal, overweight, obese and severely obese), diabetes (yes, no), age (continuous), gender (male or female), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American, and Others), education level (less than high school, high school/GED, higher than high school), and socioeconomic status (poor, not poor) was assessed for a relationship with CRS. After this, multivariate analysis through logistic regression was used to study the relationship of the different inflammatory markers with CRS. The first two questions addressed the bidirectional impact of inflammatory markers in the specific subtypes CRS through logistic regression, studying the interaction effect of each inflammatory marker with the respective independent variable of interest (CKD or CVD). The next question addressed if inflammatory markers have an independent, additive effect on CRS. Finally, the last question addressed the differential impact of race and socioeconomic status on inflammation and CRS. These four questions was

addressed through multiple regression, controlling for confounding factors like obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status.

Data Collection and Analysis

The purpose of this study was to evaluate the relationship between inflammatory markers and CRS. More specifically, the research questions that guided this study were:

Research Question 1

Do elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) modify the effect of CKD on CVD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o1. The elevated inflammatory biomarkers do not modify the effect of CKD on CVD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

H_a1. The elevated inflammatory biomarkers do modify the effect of CKD on CVD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

In order to address this research question simulating Type 4 CRS, a stepwise logistic regression model was utilized to assess the association between the dependent variable cardiovascular disease (Y) and CKD (X_i) while controlling for selected covariates (X_{ic}) such as obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Preliminarily, the model significance levels was set at levels that are greater than 0.05. For inclusion in the model, as Menard (1997) recommends, p=0.10 was utilized and for exclusion from the model p=0.15 was utilized in order to ensure that variables are not

prematurely eliminated stepwise regression model. For the final test of statistical significance, a two-tailed p-value <0.05 or 95% Cl of the odds ratio that excludes 1.0 was considered significant. In the final model, first order interactions were assessed in order to test for effect modification between the different inflammatory markers and CKD. Equations 1-4, written below, reflect the regression models being studied. Using the Hosmer and Lemeshow test, a model would pass the goodness-of-fit if p>0.05. Additionally, multicollinearity was assessed by testing the final model in logistic regression with >0.20 tolerance level (Tabachnick & Fidell, 2014). The equations would be as follows:

1. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*CKD + β_{78}*hcy_{high}

   Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*CKD + β_{78}*hcy_{Low}
2. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*CKD + β_{78}*F/T_{high}
3. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*CKD + β_{78}*F/T_{Low}
4. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*CKD + β_{78}*$fibrinogen_{high}$

5. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*CKD + β_{78}*$\text{fibrinogen}_{\text{Low}}$
6. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*CKD + β_{78}*$\text{hs-CRP}_{\text{high}}$
7. Logit (CVD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*CKD + β_{78}*$\text{hs-CRP}_{\text{Low}}$

Research Question 2

Do elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) modify the effect of CVD on CKD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o2. The elevated inflammatory biomarkers do not modify the effect of CVD on CKD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

H_a2. The elevated inflammatory biomarkers do modify the effect of CVD on CKD controlling for CVD and CKD risk factors (obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status).

In a cross sectional analysis, to model the effect of CVD on CKD as is seen in Type 2 CRS, the following method was used. A backward stepwise logistic regression model was utilized to assess the association between the dependent variable CKD (Y) and CVD

(X_i) while controlling for selected covariates (X_{ic}) such as obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Preliminarily, the model significance levels was set at levels that are greater than 0.05. For inclusion in the model, as Menard (1997) recommends, p=0.10 was utilized and for exclusion from the model p=0.15 was utilized in order to ensure that variables are not prematurely eliminated stepwise regression model. For the final test of statistical significance, a two-tailed p-value <0.05 or 95% Cl of the odds ratio that excludes 1.0 was considered significant. In the final model first order interactions was assessed in order to test for effect modification between the different inflammatory markers and CVD. Equations 5-8, written below, reflect the regression models being studied. Using the Hosmer and Lemeshow test a model would pass the goodness-of-fit if p>0.05. Additionally, multicollinearity was assessed by testing the final model in logistic regression with >0.20 tolerance level (Tabachnick & Fidell, 2014). The equations would be as follows:

8. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*CVD + β_{78}*hcy_{high}
9. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*CVD + β_{78}*hcy_{Low}
10. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*CVD + β_{78}*F/T_{high}
11. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*CVD + β_{78}*F/T_{Low}

12. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*CVD + β_{78}*$\text{fibrinogen}_{\text{high}}$
13. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*CVD + β_{78}*$\text{fibrinogen}_{\text{Low}}$
14. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*CVD + β_{78}*$\text{hs-CRP}_{\text{high}}$
15. Logit (CKD) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*CVD + β_{78}*$\text{hs-CRP}_{\text{Low}}$

Research Question 3

Do elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) have an additive effect on CRS along with CVD risk factors (e.g. obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o3. Elevated inflammatory biomarkers do not act as additive risk factors and increase the susceptibility of CRS along with known CVD risk factors.

H_a3. Elevated specific inflammatory biomarkers do act as additive risk factors of CRS along with known CVD risk factors.

In order to test for the direct effect of inflammatory marker on CRS, a backward stepwise logistic regression model was utilized to assess the association between the

dependent variable CRS (Y) and inflammatory markers (i.e. hcy, F/T, fibrinogen, and hs-CRP) (X_i) while controlling for selected covariates (X_{ic}) such as obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Equations 9-12, written below, reflect the regression models being studied. Preliminarily, the model significance levels was set at levels that are greater than 0.05. For inclusion in the model, as Menard (1997) recommends, p=0.10 was utilized and for exclusion from the model p=0.15 was utilized in order to ensure that variables are not prematurely eliminated stepwise regression model. For the final test of statistical significance, a two-tailed p-value <0.05 or 95% Cl of the odds ratio that excludes 1.0 was considered significant. Using the Hosmer and Lemeshow test a model would pass the goodness-of-fit if p>0.05. Additionally, multicollinearity was assessed by testing the final model in logistic regression with >0.20 tolerance level (Tabachnick & Fidell, 2014). The equations would be as follows:

16. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy
17. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T
18. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen
19. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP

Research Question 4

Do sociodemographic (race/ethnicity, family income, expressed relative to the poverty threshold, or education level) indicators play a modifying role between the relationship of inflammatory markers and CRS controlling for known CVD risk factors (e.g. obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status)?

H_o4. Sociodemographic indicators do not play a modifying role between the relationship of inflammatory markers and CRS.

H_a4. Sociodemographic indicators do play a modifying role between the relationship of inflammatory markers and CRS.

In a cross sectional analysis, to model the effect of demographic factors on the relationship between inflammatory markers and CRS, the following method was used. A backward stepwise logistic regression model was utilized to assess the association between the dependent variable CRS (Y) and demographic factors (race/ethnicity, family income, or education level) (Xi) while controlling for selected covariates (Xic) such as obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Preliminarily, the model significance levels was set at levels that are greater than 0.05. For inclusion in the model, as Menard (1997) recommends, p=0.10 was utilized and for exclusion from the model p=0.15 was utilized in order to ensure that variables are not prematurely eliminated stepwise regression model. For the final test of statistical significance, a two-tailed p-value <0.05 or 95% Cl of the odds ratio that excludes 1.0 was considered significant. In the final model first order interactions was assessed in order to test for effect modification between the individual inflammatory markers and individual

demographic factors. Equations 13-24, written below, reflect the regression models being studied. Using the Hosmer and Lemeshow test a model would pass the goodness-of-fit if $p>0.05$. Additionally, multicollinearity was assessed by testing the final model in logistic regression with >0.20 tolerance level (Tabachnick & Fidell, 2014). The equations would be as follows:

20. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*$\text{race}_{\text{Non-Hispanic White}}$
21. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*$\text{race}_{\text{Non-Hispanic Black}}$
22. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*$\text{race}_{\text{Hispanic}}$
23. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*F/T + β_8*$\text{race}_{\text{Non-Hispanic White}}$
24. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*F/T + β_8*$\text{race}_{\text{Non-Hispanic Black}}$
25. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*F/T + β_8*$\text{race}_{\text{Hispanic}}$
26. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*$\text{race}_{\text{Non-Hispanic White}}$
27. Logit (CRS) = $\beta_0 + \beta_1$*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*$\text{race}_{\text{Non-Hispanic Black}}$

28. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*race$_{\text{Hispanic}}$
29. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*race$_{\text{Non-Hispanic White}}$
30. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hs-CRP + β_8*race$_{\text{Non-Hispanic Black}}$
31. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*race$_{\text{Hispanic}}$
32. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*family income$_{\text{High}}$
33. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hcy + β_8*family income$_{\text{Low}}$
34. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*F/T + β_8*family income$_{\text{High}}$
35. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*F/T + β_8*family income$_{\text{Low}}$
36. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*family income$_{\text{High}}$
37. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*fibrinogen + β_8*family income$_{\text{Low}}$
38. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*family income$_{\text{High}}$

39. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7*hs-CRP + β_8*family income$_{\text{Low}}$
40. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hcy + β_8*education level$_{\text{some high school}}$
41. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hcy + β_8*education level$_{\text{high school graduate}}$
42. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hcy + β_8*education level$_{\text{some college}}$
43. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*education level$_{\text{some high school}}$
44. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*education level$_{\text{high school graduate}}$
45. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* F/T + β_8*education level$_{\text{some college}}$
46. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* fibrinogen + β_8*education level$_{\text{some high school}}$
47. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* fibrinogen + β_8*education level$_{\text{high school graduate}}$

48. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* fibrinogen + β_8*education level$_{\text{some college}}$
49. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hs-CRP + β_8*education level$_{\text{some high school}}$
50. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hs-CRP + β_8*education level$_{\text{high school graduate}}$
51. Logit (CRS) = β_0 + β_1*obesity + β_2*age + β_3*hypercholesterolemia + β_4*gender + β_5*diabetes status + β_6*smoking status + β_7* hs-CRP + β_8*education level$_{\text{some college}}$

Sample Weights and Other Considerations

Due to a multistage, complex, probability cluster design, there has to be special techniques utilized to decrease the correlation amount that is found within a specific cluster (CDC, 2011). This specific design requires for the use of differential weighting, stratification, and clustering. Logistically, in order to achieve this, fewer individuals with a cluster and multiple clusters are sampled. A total of 30 population sampling units (PSUs) are sampled within a 2-year period (CDC, 2011). Consequently, one PSU represents 5,000 individuals. Individual sample weights are placed on individual subjects who are part of an oversampled (downweighted) or undersampled group (Park & Lee, 2004). Due to this specialized sampling technique, if simple random sampling is used,

then the significance levels would be overstated and variance estimates would be too low due to the lack of consideration of differential weighting (CDC, 2011).

In order to derive the weighing amounts certain calculations were made first. Three steps of calculations are made for sample weights (CDC, 2013). First of all, the final probability by calculating the product of the probability of an individual being selected, the probability of the household being selected, the probability of the section of the PSU being selected, and the probability of the PSU being selected (CDC, 2013). This then had to be adjusted for nonresponse. The final adjustment that is made is the post-stratification adjustment for the purpose of matching the control totals derived from the year 2000 United States Census population. In order to understand the use of sample weights, the design effect (DEFF) is calculated. The DEFF is derived by finding the ratio of the variance of the complex sample design by the simple random sample (CDC, 2011). Usually, in NHANES the DEFF is greater than 1, or the variance estimate of the cluster is greater than that of a simple random sample. The DEFF varies for different variables, and the minimum sample size for analysis should yield a relative standard error of 30% or less (CDC, 2011). In order to take into account the complex sample design, the Taylor Series Linearization was used. Because of ethical issues, actual PSUs cannot be used, and Masked Variance Units (MVUs) are used. While the PSU variable is known as sdmvpsu in the dataset, the stratum variable is known as sdmvstra (CDC, 2009). Additionally, the individual weighting previously mentioned has the prefix “wt” (CDC, 2009).

The NHANES study is a study which is representative of the national population. Researchers in the Centers for Disease Control and Prevention estimated that each participant corresponds to approximately 50,000 residents in the United States (CDC, 2013b). In order to simplify the process, the U.S. was divided into different counties, which was further divided into local areas, which was divided into housing communities, and then divided into households. In order to maintain randomness, each year 15 different counties are sampled randomly. Each individual household is also selected randomly. Random sampling serves two purposes: it ensures generalizability of the findings from research and it ensures that equal probabilities of subjects participate in the survey.

Sampling Method

There are numerous measures the National Center for Health Statistics (NCHS) takes in order to ensure a high rate of participation and optimal quality of results (CDC, 2013b). Through local media personnel, the NCHS informs the individuals of the local communities about the details of the upcoming study and notifies the leadership in the specific community. An introductory letter is sent to each participant in order to explain the details of what the survey entails and the benefits. In order to ensure that each participant is eligible, the interviewers from NCHS visit each individual household. The participants are guaranteed confidentiality, informed of their rights to stop participation when they choose, instructed about the goals and purpose of the survey, and the different components of the NHANES survey. Along with the household interview, NCHS workers about conduct medical examination in mobile examination centers (CDC,

2013b). Consequently, participants are given a written consent for the household interview and one for the medical examination. If the participant chooses to, they can participate in one without participating in the other component.

Ethical Protection of Human Participants

Different ethical guidelines must be considered in order to perform research studies with human subjects. Before data collection for NHANES, the NCHS received approval from the NCHS Research Ethics Review Board (changed from the Institute Review Board), continuance of the protocol #2011-17. The NCHS complies strictly with the different laws and regulations which are written with the intent of protecting the specific participant's confidentiality and safety (McQuillan & Porter, 2011). Other protective laws that are in place are the Public Health Service Act of 1956 (42 USC 242k) which allows for the collection of data and the Privacy Act of 1974 (5 USC 552A) (Pappas & Hyder, 2005). Finally, the Confidential Information Protection and Statistical Efficiency Act (PL 107-347) prohibits the illegal information distribution which could lead to subject identification without the participant's expressed consent or permission. The NCHS ensures that the collected data is stored securely with all personally identifying information replaced with codes, making it impossible to retrace the subjects back. Because there is high variability of literacy and health literacy, the NCHS takes extra measures in order to ensure that the consent is written at a literacy level that the majority of the population can understand.

This study used publicly available secondary data provided by the NCHS. Before collection of data, informed consent was obtained for the home interview and

medical examination components. During the examination component, the subjects wear bar code bracelets so that the examiners are even blinded to the personal identifier. After collection of data, specific personal identifiers like social security number, name, email address, and birthdate are removed before publication of data. Because this study cannot be traced or linked in any way back to the individual participant, the study poses no violation of confidentiality to the subject. An approval by the Institute Review Board from Walden University was pursued prior to any data being gathered from the NHANES database and prior to data analysis in order to meet additional ethical requirements. The IRB approval number is 05-27-14-0303779. Additionally, the sole intent of this study was to fill knowledge gaps without seeking any financial benefits, and there is no financial interest that was involved in this study.

Summary

The NHANES data, collected between the years 1999-2010, was analyzed for this study using quantitative data analysis to evaluate the theory that elevated inflammatory markers, which are caused by several different factors, independently increase the risk of cardiovascular disease among in the United States, aged 20 years and older. By analyzing the same NHANES data sets, elevated levels of inflammatory markers was studied as to how they can independently and additively predict CRS. Chapter 4 will present the descriptive and multivariate data analyzed from NHANES, between 1999-2010. The hypotheses were evaluated to show how elevated inflammatory markers can independently and additively predict CRS in a cross-sectional manner. Chapter 5 will discuss the results presented in Chapter 4. Chapter 5 will explain how these results

promote positive social change, including the limitations of the data analysis and the recommendations for further study.

Chapter 4: Results

Introduction

The primary aim of this study was to determine whether inflammatory markers could be a potential risk factor for CRS. Additionally, by analyzing NHANES data this study evaluated the theory that elevated inflammatory markers, which are caused by smoking, obesity, high serum cholesterol levels, and hypertension independently increase the risk of CVD, CKD, and CRS in the United States, in those individuals ages 20 years and older. The distribution of each inflammatory marker is further delineated.

A representative sample of the adult U.S. population between 1999 and 2010 was examined to determine whether inflammatory markers were associated with the susceptibility to CRS. The associations between CRS and other variables, age, gender, poverty level, education, marital status, and diabetes were also explored during bivariate analyses. Age was divided into seven categories representing each decade of life: 20-29; 30-39; 40-49; 50-59; 60-69; 70-79; and ≥80. Socioeconomic factors and variables that were determined to be statistically significant during bivariate analyses were further tested by logistic multivariate regression analysis. Weights adjusting for the complex NHANES design were used in all analyses to represent the U.S. population as recommended by NHANES. This chapter provides the results of these analyses.

A total of 32,464 participants were included in the NHANES 1999-2010. Among those participants, 1,872 individuals did not participate in the questionnaire. Consequently, 30,592 responded to the CVD questions, 28,278 were included in the

analysis for CKD, and 24,625 were included in the analysis for CRS. Because this study was interested in the adult population and inflammatory markers vary between children and adults, only participants between 20 and 85 years of age were included in the analyses. In addition, this study only included participants who had complete records for all tested variables. The final sample size used for analyses was 3,512 for self-reported CVD, 3,028 for CKD, and 1,548 for CRS.

Cardiovascular Disease

Demographic Characteristics

Table 2 provides data on the distribution of demographic characteristics of the participants by status of CRS using bivariate analysis. The prevalence of CVD in the US population in the age group between 20-85 years was 8.7% (n = 3,512) which is representative of 17,967,979 individuals of the United States population. The average age of participants with CVD was 64.9 ± 0.39. There was statistically significant ($p<0.001$) association between gender, age, race/ethnicity, and CVD. There were with slightly more males (52.6%) with CVD as seen in Figure 4, more Non-Hispanic Whites (77.6%), and the highest percentage over 80 years of age as seen in Figure 5.

Table 2

Risk of CVD by Demographic Characteristics

	% of Sample	CVD	*p* values
Total population n (%)		3512 (8.7)	
Age Mean (*SE*)*		64.9 (0.39)	0.0000
Age groups			
20-39 (%)	4.7	1.1	
40-59 (%)	28.6	6.5	
60-69 (%)	23.8	18.6	
70-79 (%)	24.2	27.4	
≥80 (%)	18.7	38.3	
Gender (%)*			0.000
Male	52.6	9.5	
Female	47.4	7.9	
Race/Ethnicity (%)*			0.0000
NH White	77.6	9.6	
NH Black	11.4	8.9	
Hispanic	6.6	4.5	
Other	4.3	6.9	

Note. *Tests for difference were statistically significant

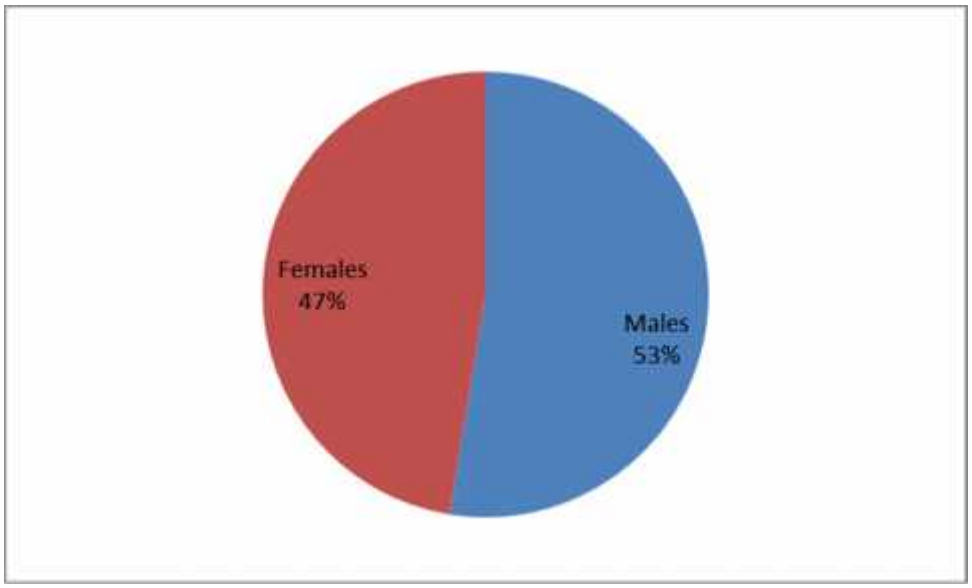

Figure 4. Gender distribution of CVD.

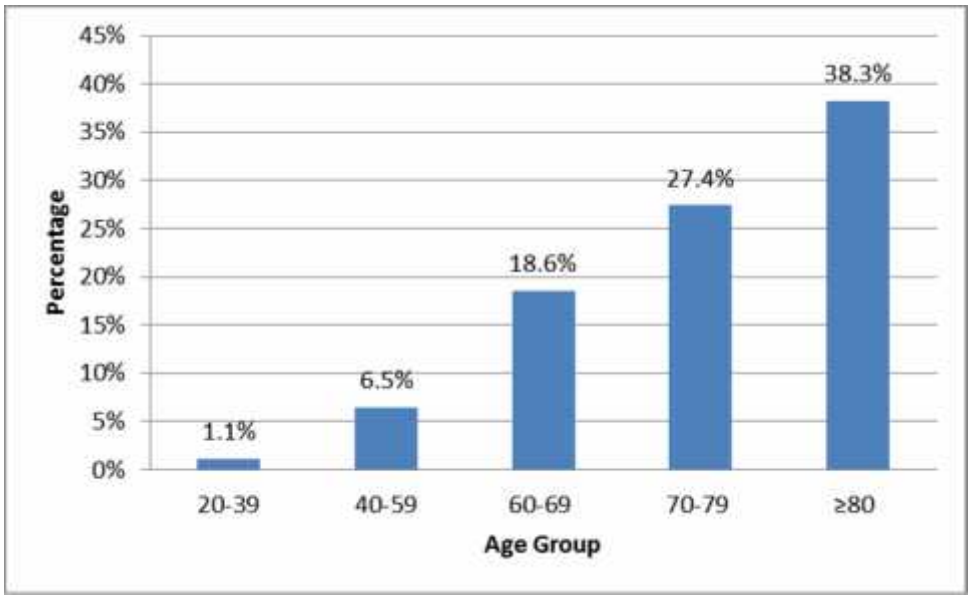

Figure 5. Percentage of individuals in each age group with CVD (N=3512).

CVD and Covariates

In addition to the basic demographic descriptive statistics, the following variables that may confound the effect and modifying effect of inflammatory biomarkers on

CVD are presented in Table 3. Based on bivariate analyses, individuals with less than a high school education had the highest percentage rates (13.3%) of CVD. Individuals in lower socioeconomic status had CVD (10.7%) than those individuals in higher economic status (8.3%). Finally, more individuals with diabetes (26.1% vs 6.7%) and obesity (11.4% severely obese vs. 5.9% normal weight) were likely to have CVD than those without diabetes and obesity. When the Framingham Risk Score was calculated, the group with CVD had a significantly higher mean risk score than those without CVD (14.7 vs 12.8, $p < 0.001$).

Table 3

Risk of CVD Across Covariates

Variable	% of sample	CVD	*p* values
Education (%)*			0.0000
Less than HS	30.3	13.3	
HS	28.9	10.1	
Higher than HS	40.8	6.5	
PIR (%)*			0.0000
Poor	16.8	10.7	
Not poor	83.2	8.3	
Diabetes (%)*			0.0000
Yes	27.9	26.1	
No	72.1	6.7	
BMI Mean (*SE*)		29.8 (.14)	0.001
BMI Groups*			
Normal (<25)	23.0	5.9	0.0000
Overwt. (25-	34.7	8.7	
Obese (30-39.9)	34.9	10.7	
S. Obese (>40)	7.4	11.4	
High Cholesterol			
Yes	64.3	17.9	
No	35.7	6.9	
Smoking Status (%)			0.001
Current Smoker	20.0	7.4	
Former Smoker	41.2	14.5	
Nonsmoker	38.8	6.5	
Hypertension (%)			
Yes	62.5	23.3	0.0000
No	37.5	4.3	
Framingham Risk Score Mean (SE)*		14.8 (.14)	<.001

Note. * Tests for difference among the groups were statistically significant ($p \leq 0.001$).

Chronic Kidney Disease

Demographic Characteristics

Table 4 provides data on the distribution of demographic characteristics of the participants by status of CKD using bivariate analysis. The prevalence of CKD in US population in the age group between 20-85 was 7.1% (n = 3,028) which is representative of 13,797,140 individuals of the United States population. The average age of participants with CKD was 74.4 ± 0.28. There was statistically significant ($p<0.001$) association between gender, age, race/ethnicity, and CKD. There were with significantly more females (68.0%) with CKD than males as seen in Figure 6. According to Figure 7, most (73.6%) of the individuals who were age 80 and above had CKD. Among Non-Hispanic Whites, the percentage (8.1%) with CKD was the highest.

Table 4

Risk of CKD by Demographic Characteristics

	% of Sample	CKD	*p* values
Total population n (%)		3028 (7.1)	
Age Mean (*SE*)*		74.4 (0.28)	0.0000
Age groups			
20-39 (%)	0.9	0.2	
40-59 (%)	8.1	1.5	
60-69 (%)	13.	8.6	
70-79 (%)	36.	34.	
≥80 (%)	41.	73.	
Gender (%)			0.0000
Male	32.	4.7	
Female	68.	9.4	
Race/Ethnicity (%)*			0.0000
NH White	80.	8.1	
NH Black	9.3	6.3	
Hispanic	5.2	2.9	
Other	5.0	6.6	

* Tests for difference were statistically significant

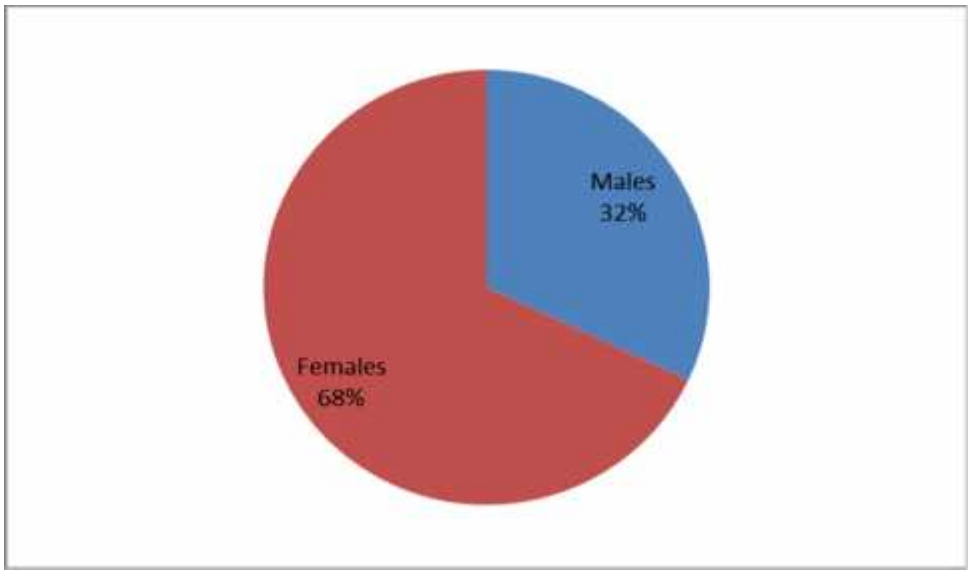

Figure 6. Gender distribution of CKD.

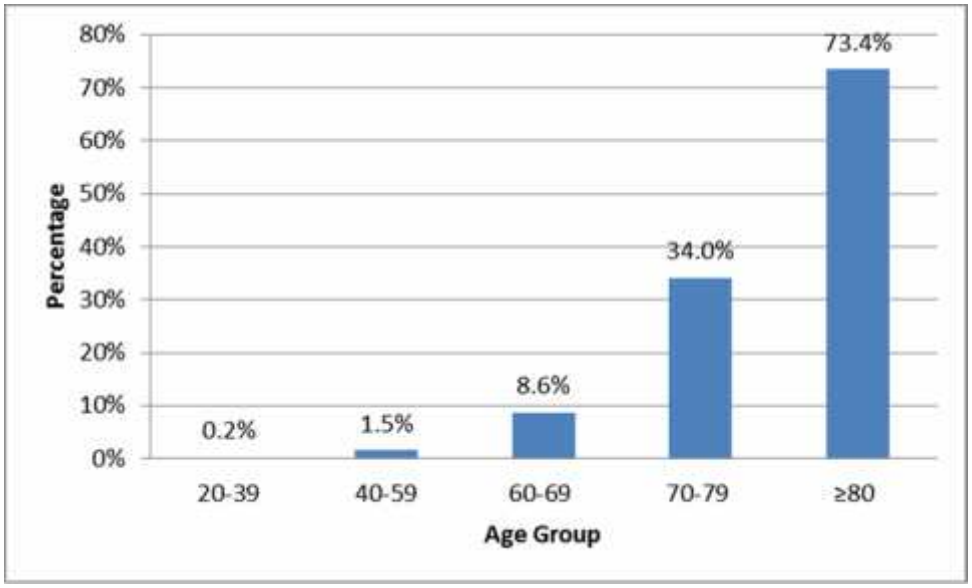

Figure 7. Percentage of individuals in each age group with CKD (N=3028)

CKD and Covariates

In addition to the basic demographic descriptive statistics, the following variables that may confound the effect and modifying effect of inflammatory biomarkers on

CKD are presented in Table 5. Based on bivariate analyses, individuals with less than a high school education had the highest percentage rates (10.4%) of CKD. Individuals in lower socioeconomic status had CKD (9.6%) than those individuals in higher economic status (5.7%). Finally, more individuals with diabetes (14.7% vs 6.3%) and hypertension (23.3% vs. 4.3%) were likely to have CKD than those without diabetes and hypertension.

Table 5

Risk of CKD Across Covariates

Variable	% of sample	CKD	*p* values
Education (%)*			0.0000
Less than HS	28.	10.	
HS	27.	7.9	
Higher than HS	44.	5.7	
PIR (%)*			0.0000
Poor	46.	9.6	
Not poor	54.	5.7	
Diabetes (%)*			0.0000
Yes	18.	14.	
No	81.	6.3	
BMI Mean (*SE*)		25.2 (.12)	0.0000
BMI Groups*			
Normal (<25)	52.	11.	0.0000
Overwt. (25-	33.	6.9	
Obese (30-39.9)	13.	3.4	
S. Obese (>40)	1.1	1.4	
High Cholesterol (%)			0.0000
Yes	64.	12.	
No	35.	6.5	
Smoking Status (%)			0.000
Current Smoker	11.	3.5	
Former Smoker	36.	10.	
Nonsmoker	52.	7.2	
Hypertension (%)			
Yes	62.	23.	0.0000
No	37.	4.3	

* Tests for difference among the groups were statistically significant ($p \leq 0.001$).

Cardiorenal Syndrome

Demographic Characteristics

Table 5 provides data on the distribution of demographic characteristics of the participants by status of CRS using bivariate analysis. The prevalence of CRS in US population in the age group between 20-85 was 3.9% (n=1,548) which is representative of 6,770,775 of the United States population. The average age of participants with CRS was 72.8 ± 0.41. There was no statistically significant difference association between gender and CRS, with slightly more females (according to Figure 8) with CRS and more Caucasians (78.7%). According to Figure 9, most (63.8%) of the individuals who were age 80 and above had CRS. Age and race/ethnicity tested statistically significant ($p<0.05$).

Table 6

Risk of CRS by Demographic Characteristics

	% of Sample	CRS	*p* values
Total population n (%)		1548 (3.9)	
Age Mean (*SE*)*		72.8 (0.41)	0.0000
Age groups			
20-39 (%)	0.7	0.1	
40-59 (%)	11.	1.1	
60-69 (%)	18.	7.0	
70-79 (%)	32.	23.	
≥80 (%)	37.	63.	
Gender (%)			0.652
Male	48.	3.8	
Female	51.	4.0	
Race/Ethnicity (%)*			0.0000
NH White	78.	4.4	
NH Black	11.	4.3	
Hispanic	5.8	1.7	
American			
Other	3.8	2.8	

* Tests for difference were statistically significant

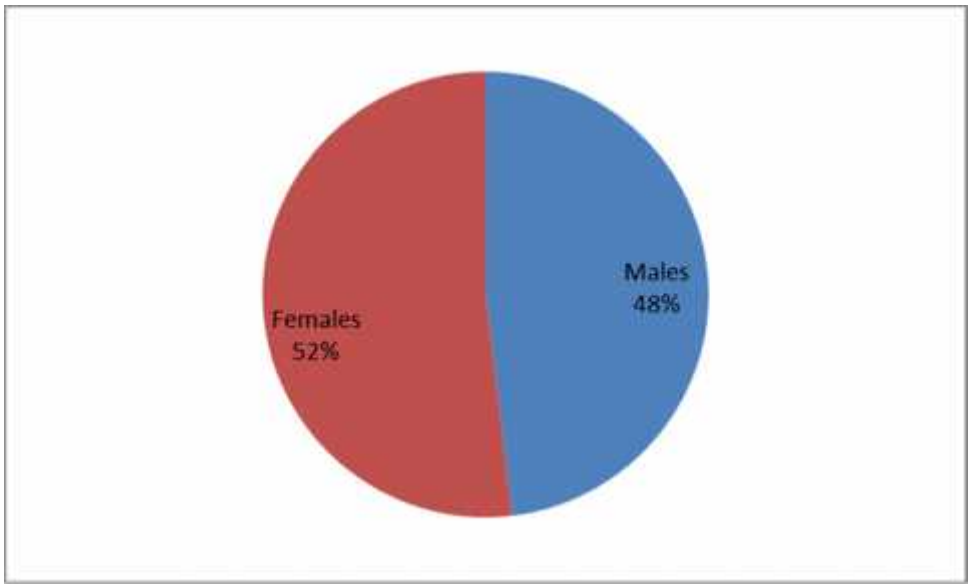

Figure 8. Gender distribution of CRS.

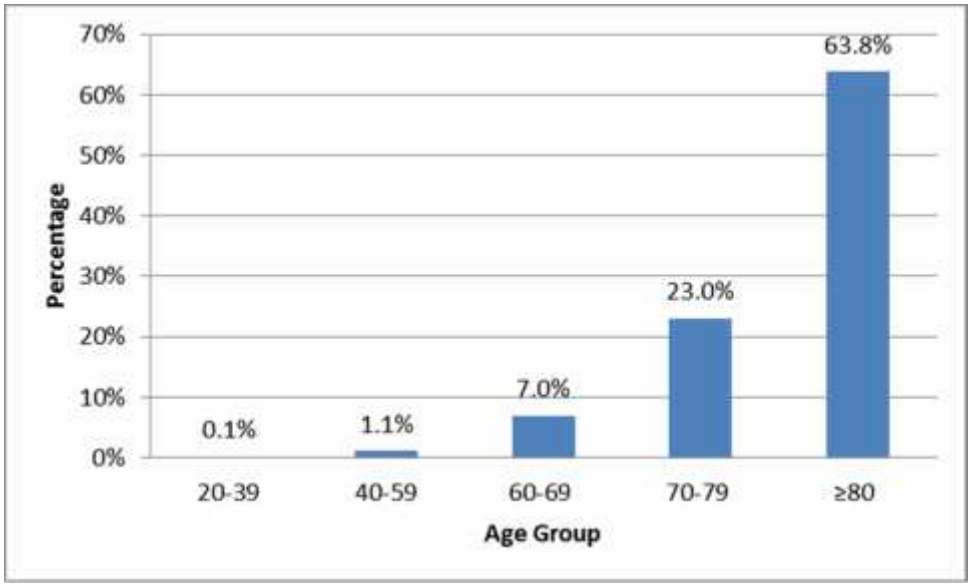

Figure 9. Percentage of individuals in each age group with CRS (N=1548)

CRS and Covariates

In addition to the basic demographic descriptive statistics, the following variables that may confound the effect and modifying effect of inflammatory biomarkers on CRS are presented in Table 7. Based on bivariate analyses, individuals with less than a high school education had the highest percentage rates (7.0%) of CRS. Individuals in lower socioeconomic status had CRS (4.3%) than those individuals in higher economic status (3.7%). Finally, more individuals with diabetes (24.2% vs 6.6%) and hypertension (27.1% vs. 4.7%) were likely to have CRS than those without diabetes and hypertension.

Table 7

Risk of CRS Across Covariates

Variable	% of sample	CRS	*p* values
Education (%)*			0.0000
Less than HS	33.	7.0	
HS	26.	4.2	
Higher than HS	39.	2.7	
PIR (%)			0.08
Poor	14.	4.3	
Not poor	85.	3.7	
Diabetes (%)*			0.0000
Yes	59.	24.	
No	41.	6.6	
BMI Mean (*SE*)		28.4 (.19)	0.0000
BMI Groups*			
Normal (<25)	34.	12.	< 0.05
Overwt. (25-	38.	9.3	
Obese (30-39.9)	23.	7.5	
S. Obese (>40)	3.6	11.	
High Cholesterol (%)			
Yes	60.	16.	
No	39.	11.	
Smoking Status (%)			0.001
Current Smoker	18.	8.0	
Former Smoker	36.	16.	
Nonsmoker	45.	8.6	
Hypertension (%)			
Yes	66.	27.	0.0000
No	33.	4.7	

* Tests for difference among the groups were statistically significant ($p \leq 0.001$).

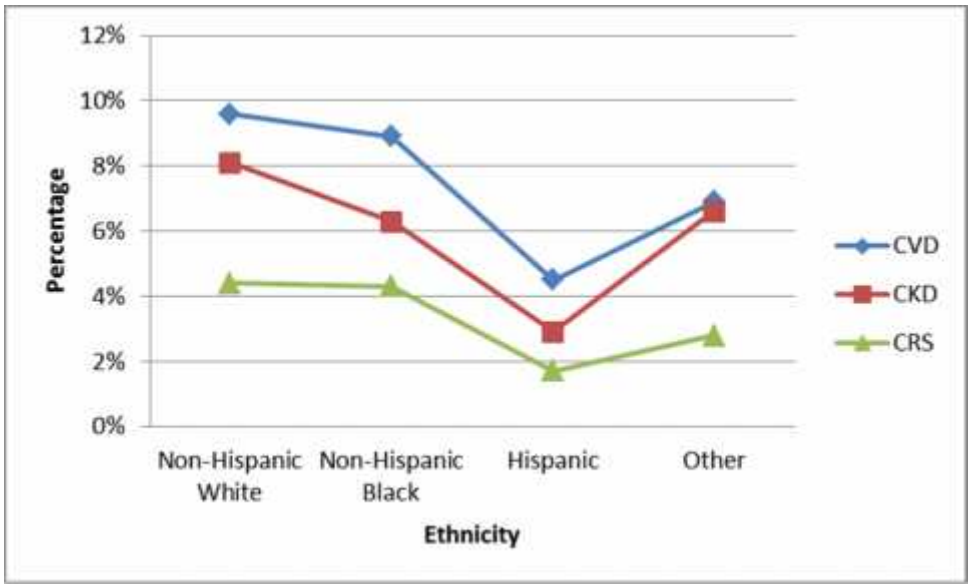

Figure 10. Percentage of individuals in each ethnicity with CVD, CKD, or CRS

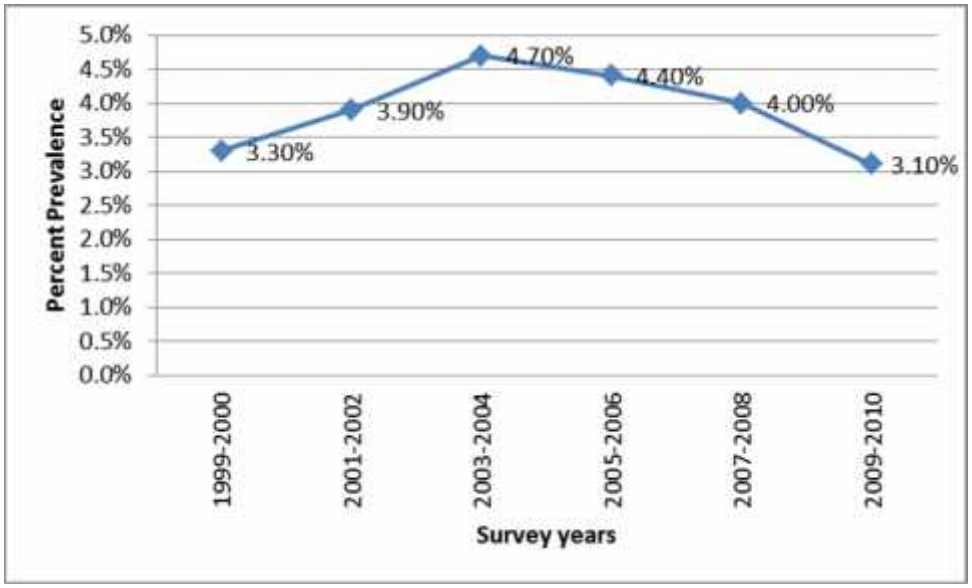

Figure 11. Prevalence of cardiorenal syndrome between 1999-2010

The percentage distribution of CVD, CKD, and CRS is shown in Figure 10. This demonstrates that the percentage of CRS in Non-Hispanic Whites and Non-Hispanic Blacks is almost equal. Additionally, the prevalence of CRS in each biannual period

was tracked in the NHANES years 1999-2010 in Figure 11. Cardiovascular health among non-institutionalized individuals aged 20 years and older for NHANES years 1999-2010 is shown in Table 8. Cardiovascular health for this evaluation includes congestive heart failure, coronary heart disease, angina, stroke and heart attack. All reports were based on whether or not the study participant was informed by a physician if she has had one or more of these conditions. Out of all individuals, 8.7% of the study population reported being told by a physician that they have had either congestive heart failure (2.3%), coronary heart disease (3.4%), angina (2.6%), stroke (2.7%), and heart attack (3.4%). The remaining approximately 91.3% of the study population either have not been told by a physician that she had either one or more of these heart conditions or data was missing from these individuals.

Table 8

Cardiovascular Health of Individuals Aged 20 Years and Older, NHANES, 1999-2010

	N	%
Congestive Heart Failure	1003	2.3
Coronary Heart Disease	1307	3.4
Angina	984	2.6
Stroke	1154	2.7
Heart Attack	1387	3.4

Inflammatory Biomarkers

Table 9 demonstrates the mean and standard error of the original continuous variables and the years the measurements were made. As demonstrated in the table the mean hs-CRP was 0.42 mg/dl. Because there were not as many subjects with a fibrinogen measurement, the standard error was higher among these subjects.

Table 9

Mean and Standard Error of each inflammatory marker.

Inflammatory Biomarkers	N	Mean ± SE	Years Collected
hs-CRP	28,924	0.42 ± 0.006	1999-2010
Homocysteine	9,453	8.76 ± 0.086	2001-2006
Fibrinogen	3,010	372.97 ± 4.768	2001-2002
F/T Ratio	5,943	20.51 ± 0.364	1999-2010 (females age 20-49)

More univariate analyses were performed to examine if there was a relationship between inflammatory biomarkers and CVD. First of all, the relationship between inflammatory biomarkers and CVD was examined individually by stratifying the study sample into "elevated" and "normal" and comparing the two groups as seen in Table 10. Elevated hs-CRP was significantly associated (12.5% vs 5.4%, $p<.001$) with higher rates of CVD. Additionally, elevated homocysteine was significantly associated (14.3% vs 4.2%, $p<.001$) with CVD. Next, elevated fibrinogen was significantly associated (16.9% vs 9.1%, $p<.001$) with higher rates of CVD. Finally elevated ferritin/transferrin ratio was not significantly associated (2.6% vs.2.2%, $p=0.38$) with higher rates of CVD.

Table 10

Elevated Serum Biomarkers and CVD in individuals Age 20 years and older NHANES, 1999-2010

Inflammatory Biomarkers	*N*	% (SE%)	*p*-value
hs-CRP			<0.001
Elevated (>0.51 mg/dl)	1054	12.5 (0.5)	--
Normal (<0.09 mg/dl)	535	5.4 (0.3)	--
Homocysteine			<0.001
Elevated (≥8.15 µmol/L)	892	14.3 (0.9)	--
Normal (<8.15 µmol/L)	239	4.2 (0.3)	--
Fibrinogen			<0.001
Elevated (≥ 378 mg/dL)	303	16.9 (1.2)	--
Normal (< 378 mg/dL)	179	9.1 (1.2)	--
F/T Ratio			0.38
Elevated (≥ 11.67)	82	2.6 (0.4)	--
Normal (<11.67)	54	2.2 (0.4)	--

Univariate analysis was performed to examine if there was a relationship between inflammatory biomarkers and CKD. First of all, in the relationship between inflammatory biomarkers and CKD was examined individually by stratifying the study sample into “elevated” and “normal” and comparing the two groups as seen in Table 11. Elevated hs-CRP was significantly associated (7.6% vs 6.2%, p=0.001) with higher rates of CKD. Additionally, elevated homocysteine was significantly associated (14.2% vs 2.0%, p<.001) with CKD. Next, elevated fibrinogen was significantly associated (17.8% vs 7.6%, p<.001) with higher rates of CKD. Finally elevated ferritin/transferrin ratio was not significantly associated (0.6% vs.0.3%, p=0.15) with higher rates of CKD.

Table 11

Elevated Serum Biomarkers and CKD in individuals Age 20 years and older NHANES, 1999-2010

Inflammatory Biomarkers	N	% (SE%)	p-value
hs-CRP			0.001
Elevated (>0.51 mg/dl)	721	7.6 (0.3)	--
Normal (<0.09 mg/dl)	654	6.2 (0.3)	--
Homocysteine			<0.001
Elevated (≥8.15 µmol/L)	996	14.2 (0.7)	--
Normal (<8.15 µmol/L)	121	2.0 (0.3)	--
Fibrinogen			<0.001
Elevated (≥ 378 mg/dL)	355	17.8 (1.0)	--
Normal (< 378 mg/dL)	179	7.6 (0.6)	--
F/T Ratio			0.15
Elevated (≥ 11.67)	18	0.6 (0.1)	--
Normal (<11.67)	9	0.3 (0.1)	--

Univariate analyses were performed to examine if there was a relationship between inflammatory biomarkers and CRS. First of all, the relationship between inflammatory biomarkers and CRS was examined individually by stratifying the study sample into “elevated” and “normal” and comparing the two groups as seen in Table 12. Elevated hs-CRP was significantly associated (5.6% vs 2.3%, p<.001) with higher rates of CRS. Additionally, elevated homocysteine was significantly associated (8.2% vs 1.0%, p<.001) with CRS. Next, elevated fibrinogen was significantly associated (10.8% vs 3.2%, p<.001) with higher rates of CRS. Finally elevated ferritin/transferrin ratio was not significantly associated (0.2% vs. 0.2%, p=0.65) with higher rates of CRS.

Table 12

Elevated Serum Biomarkers and CRS in individuals Age 20 years and older NHANES, 1999-2010

Inflammatory Biomarkers	N	% (SE%)	p-value
hs-CRP			<0.001
Elevated (>0.51 mg/dl)	462	5.6 (0.3)	--
Normal (<0.09 mg/dl)	251	2.3 (0.2)	--
Homocysteine			<0.001
Elevated (≥8.15 µmol/L)	494	8.2 (0.6)	--
Normal (<8.15 µmol/L)	56	1.0 (0.2)	--
Fibrinogen			<0.001
Elevated (≥ 378 mg/dL)	166	10.8 (0.9)	--
Normal (< 378 mg/dL)	71	3.2 (0.6)	--
F/T Ratio			0.65
Elevated (≥ 11.67)	8	0.2 (0.1)	--
Normal (<11.67)	7	0.2 (0.1)	--

Hypothesis 1

Hypothesis 1 predicted that elevated inflammatory biomarkers modify the effect of CKD on CVD after controlling for known CVD risk factors like obesity, hypercholesterolemia, diabetes status, and smoking status. The demographic variables of age and gender were also controlled for so that confounding factors would not influence the study results. Initially, a multiple regression analysis was performed to determine if elevated serum CKD stages predict cardiovascular health in individuals 20 years and older within the U.S. population after controlling for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and

gender. Then, separate multiple regression analyses were run for normal versus elevated hs-CRP levels in order to look for effect modification as demonstrated in Table 13 and Figure 12. This process was repeated for homocysteine, fibrinogen, and F/T ratio.

Table 13

Age- and Sex-Adjusted CKD ORs for CVD in Multivariate Regression Modeling controlling for different demographic and CVD risk factors.

	Total OR (95% CI) (N=14,602)	CRP^- OR (95% CI) (N=3,338)	CRP^+ OR (95% CI) (N=3,732)
Model 1 (CKD)	1.28 (1.10, 1.50)*	1.08 (0.80, 1.46)**	1.51 (1.15, 2.00)
Model 2 (CKD, obesity)	1.66 (1.41, 1.96)**	1.32 (0.97, 1.79)	1.76 (1.30, 2.38)**
Model 3 (CKD, obesity, diabetes status)	1.67 (1.41, 1.99)**	1.34 (0.96, 1.87)	1.67 (1.23,2.28)**
Model 4 (CKD, obesity, diabetes status, smoking status)	1.73 (1.46, 2.07)**	1.67 (0.98, 1.93)	1.78 (1.31, 2.43)**
Model 5 (CKD, obesity, diabetes status, smoking status, hypercholesterolemia)	1.82 (1.48, 2.24)**	1.49 (1.00, 2.21)	1.92 (1.37, 2.68)**
Model 6 (CKD, Framingham Risk Score)	1.75 (1.39, 2.20)**	1.42 (0.88, 2.28)	2.40 (1.59, 3.62)**

Note. *$p < .05$ **$p < .001$

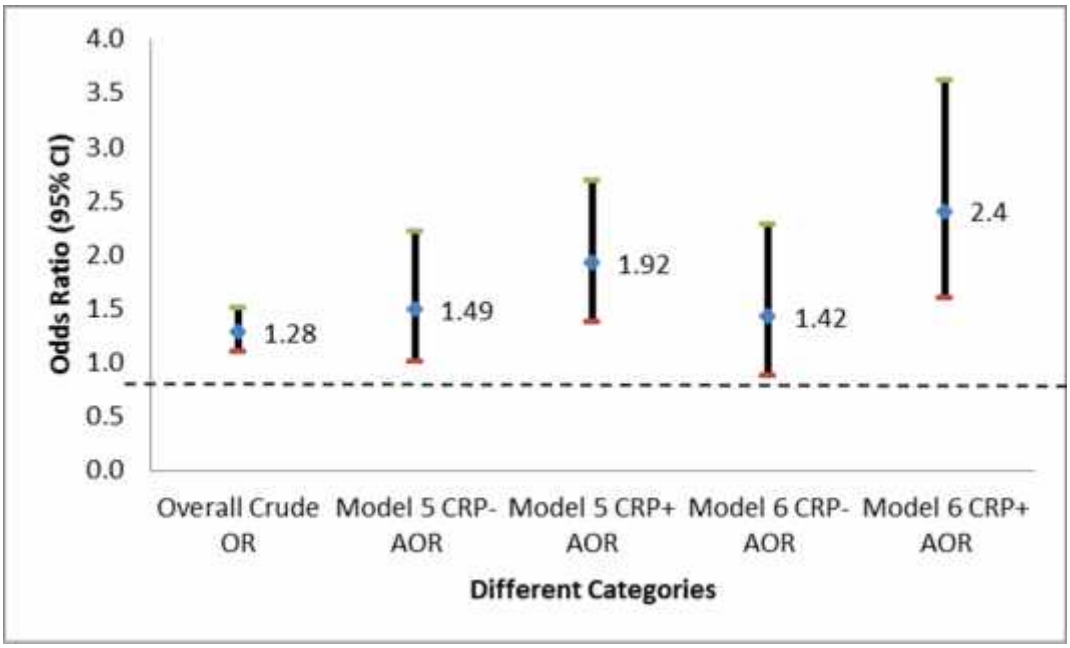

Figure 12. Crude and Adjusted Odds of CVD in individuals with CKD modified by hs-CRP level.

The overall regression model was tested at hs-CRP levels. A significant regression was found ($F(10,67) = 180.855$, $p < .001$), with the model explaining 27% (Nagelkerke R^2) of the variance and correctly classifying 89% of the cases for all variables in the model. Individuals with stage 3 CKD and higher were 1.82 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.60$, $t(76) = 5.82$, $p < .001$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and gender.

The overall regression model was tested at hs-CRP levels less than 0.09 (first quartile), representing baseline or low levels of hs-CRP. A significant regression was found ($F(10,66) = 42.750$, $p < .001$), with the model explaining 31% (Nagelkerke R^2) of

the variance and correctly classifying 93% of the cases for all variables in the model except CKD. Individuals with stage 3 CKD and higher were 1.49 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.40$, t (75) = 2.02, $p = .05$, was seen to be a statistically non-significant coefficient in the regression model. A significant regression was found for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and gender.

The overall regression model was tested at hs-CRP levels greater than 0.51 (third quartile), representing elevated levels of hs-CRP. A significant regression was found ($F(10,67) = 30.506$, $p < .001$), with the model explaining 26% (Nagelkerke R^2) of the variance and correctly classifying 85% of the cases for all variables in the model. Individuals with stage 3 CKD and higher were 1.92 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.65$, t (76) = 3.88, $p < .001$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity, diabetes status, smoking status, serum cholesterol level, as well as for demographic variables including age and gender.

Comparison of the aforementioned models demonstrates that hs-CRP plays an effect modifying role on how CKD affects CVD as hypothesized. While in the elevated hs-CRP group the odds ratio was (1.92) much higher than 1 and statistically significant, the regular hs-CRP group had a non-significant low odds ratio at 1.49 as demonstrated in Table 13 and Figure 12. The stark contrast in the odds ratio demonstrated the effect modifying role of hs-CRP. Additionally, different cofactors were significant in each hs-CRP level.

Table 14

Age- and Sex-Adjusted CKD ORs for CVD in Multivariate Regression Modeling controlling for different demographic and CVD risk factors.

	Total OR (95% CI) (N=3409)	Hcy^- OR (95% CI) (N=1653)	Hcy^+ OR (95%CI) (N=1756)
Model 1 (CKD)	1.19 (0.87, 1.62)	0.37 (0.19, 0.72)*	1.35 (0.96, 1.91)
Model 2 (CKD, obesity)	1.59 (1.16, 2.17)*	0.54 (0.28, 1.06)	1.76 (1.25, 2.49)*
Model 3 (CKD, obesity, diabetes status)	1.70 (1.18, 2.43)*	0.64 (0.29, 1.43)	1.71 (1.11, 2.62)*
Model 4 (CKD, obesity, diabetes status, smoking status)	1.80 (1.24, 2.61)*	0.64 (0.27, 1.49)	1.88 (1.21, 2.90)*
Model 5 (CKD, obesity, diabetes status, smoking status, hypercholesterolemia)	2.00 (1.32, 3.00)*	0.66 (0.27, 1.61)	2.21 (1.43-3.42)*
Model 6 (CKD, obesity, diabetes status, smoking status, hypercholesterolemia, hypertension)	1.98 (1.32, 2.97)*	0.65 (0.28, 1.53)	2.29 (1.44, 3.64)*

Note. $*p < .05$ $**p < .001$

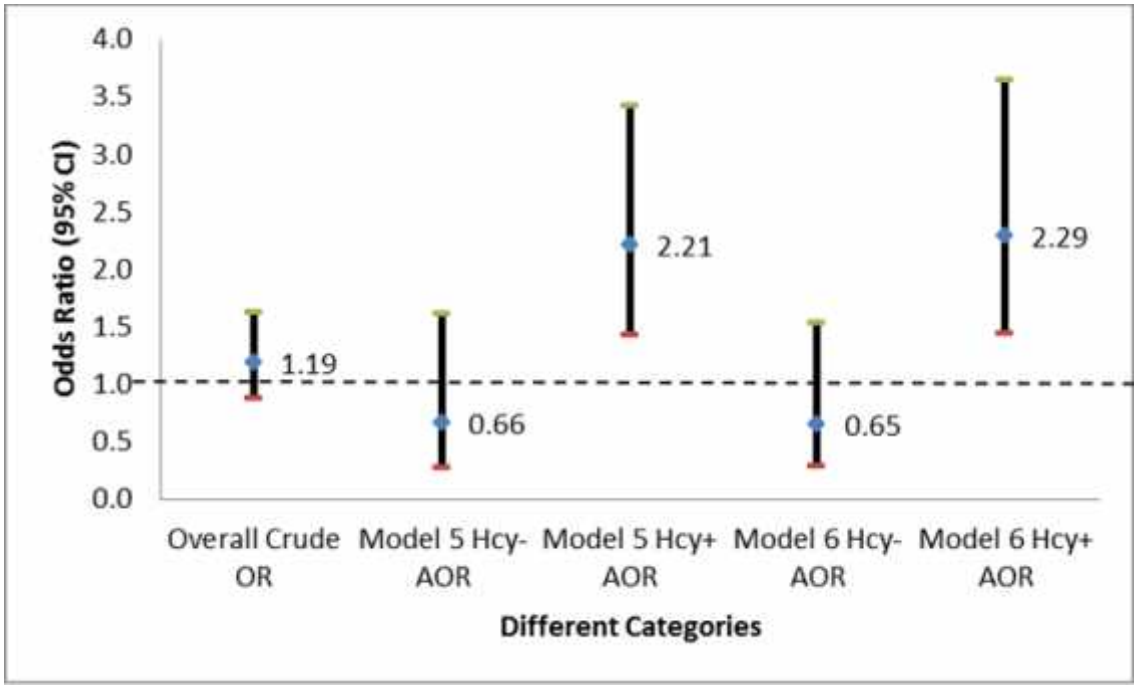

Figure 13. Crude and Adjusted Odds of CVD in individuals with CKD modified by hcy level.

The overall regression model was tested at all hcy levels as demonstrated in Table 14 and Figure 13. A significant regression was found ($F(10,21) = 47.637$, $p < .001$), with the model explaining 28% (Nagelkerke R^2) of the variance and correctly classifying 90% of the cases for all variables in the model. Individuals with stage 3 CKD and higher were 2.00 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.69$, $t(30) = 3.44$, $p = .002$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and gender.

The regression model was tested at hcy levels less than 8.15µmol/L (50^{th} percentile), representing baseline or low levels of hcy. A significant regression was found ($F(10,20) = 11.620$, $p < .001$), with the model explaining 20% (Nagelkerke R^2) of the variance and correctly classifying 95% of the cases for all variables in the model except CKD. Individuals with stage 3 CKD and higher were 0.66 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.42$, $t(29) = 0.96$, $p = .34$, was seen to be a statistically non-significant coefficient in the regression model. A significant regression was found for obesity, hypercholesterolemia, and diabetes status as well as for the demographic variable including age. Gender and smoking status were non-significant factors in the model.

The regression model was tested at hcy levels ≥ 8.15 µmol/L (50^{th} percentile), representing elevated levels of hcy. A significant regression was found ($F(10,21) = 28.359$, $p < .001$), with the model explaining 28% (Nagelkerke R^2) of the variance and correctly classifying 85% of the cases for all variables in the model. Individuals with stage 3 CKD and higher were 2.21times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.80$, $t(30) = 3.73$, $p = .001$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity, diabetes status, serum cholesterol level, smoking status as well as for demographic variables including age and gender.

Comparison of the aforementioned models demonstrates that homocysteine plays an effect modifying role on how CKD affects CVD as hypothesized and demonstrated in Table 14 and Figure 13. While in the elevated hcy group the odds ratio was (2.21) much

higher than 1 and statistically significant, the regular hcy group had a non-significant low odds ratio at 0.66. The stark contrast in the odds ratio demonstrated the effect modifying role of hcy. Additionally, different cofactors were significant in each hcy level.

Table 15

Age- and Sex-Adjusted CKD ORs for CVD in Multivariate Regression Modeling controlling for different demographic and CVD risk factors.

	(N=2827) OR (95% CI)	**Fibrinogen$^-$ OR (95% CI) (N=1424)**	**Fibrinogen$^+$ (95% CI) (N=1403)**
Model 1 (CKD)	1.53 (1.05-2.24)*	1.39 (0.79, 2.44)	1.64 (1.09-2.48)*
Model 2 (CKD, obesity)	2.24 (1.48-3.40)*	1.98 (1.21, 3.25)*	2.17 (1.47, 4.26)*
Model 3 (CKD, obesity, diabetes status)	2.21 (1.46-3.33)*	1.98 (1.22, 3.23)*	2.42 (1.41, 4.14)*
Model 4 (CKD, obesity, diabetes status, smoking status)	2.33 (1.46-3.71)*	2.11 (1.27-3.51)*	2.56 (1.45, 4.54)*
Model 5 (CKD, obesity, diabetes status, smoking status, hypercholesterolemia)	2.55 (1.40-4.66)*	2.06 (0.60-7.01)	3.16 (1.45-6.85)*
Model 6 (CKD, obesity, diabetes status, smoking status, hypercholesterolemia, hypertension)	2.43 (1.29-4.57)*	1.95 (0.56-6.81)	3.00 (1.34-6.69)*

Note. *$p < .05$ **$p < .001$

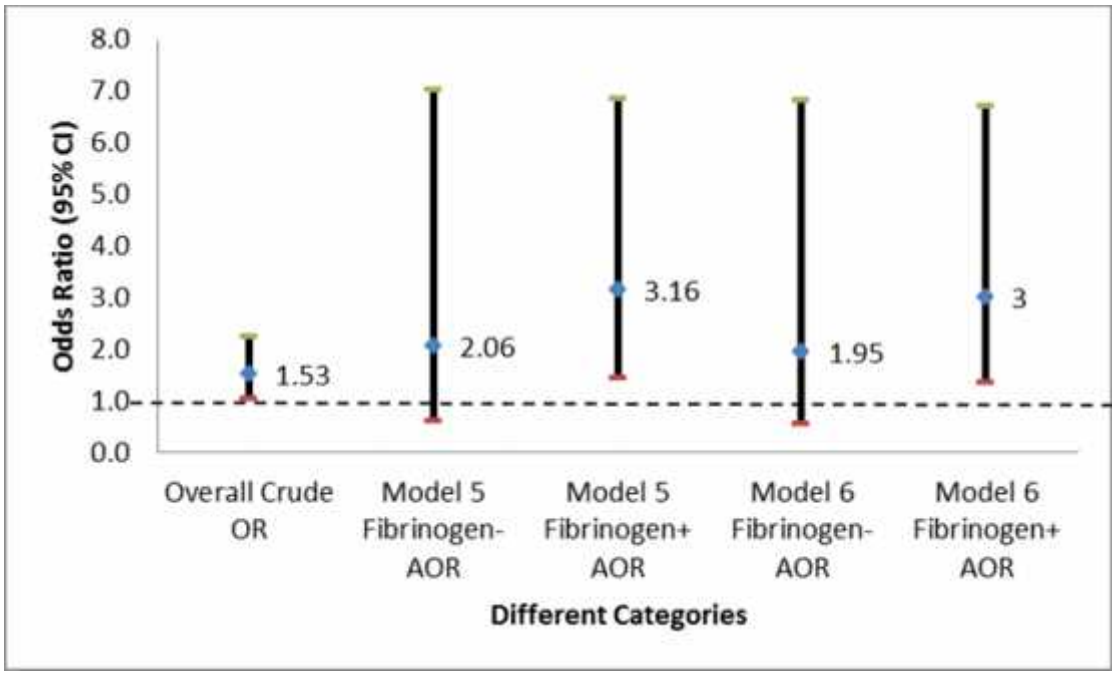

Figure 14. Crude and Adjusted Odds of CVD in individuals with CKD modified by fibrinogen level

The regression models were tested at all fibrinogen levels, normal fibrinogen levels, and elevated fibrinogen levels respectively to assess for effect modification. At all fibrinogen levels, a significant regression was found ($F(10,6) = 52.088$, $p < .001$), with the model explaining 23% (Nagelkerke R^2) of the variance and correctly classifying 78% of the cases for all variables in the model as demonstrated in Table 15 and Figure 14. Individuals with stage 3 CKD and higher were 2.55 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 0.94$, $t(15) = 3.32$, $p = .002$, was seen to be a significant coefficient in the regression model. A significant regression was

found for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and gender.

The regression model was tested at fibrinogen levels less than 378 mg/dL (50^{th} percentile), representing baseline or low levels of fibrinogen. A significant regression was found ($F(10,6) = 11.287$, $p = .004$), with the model explaining 26% (Nagelkerke R^2) of the variance and correctly classifying 82% of the cases for all variables in the model except CKD. Individuals with stage 3 CKD and higher were 0.49 times more likely to exhibit CVD than those without CKD. CKD, $\beta = 0.72$, $t(15) = 1.25$, $p = 0.23$, was seen to be a statistically non-significant coefficient in the regression model. A significant regression was found for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age. Gender, diabetes, and obesity were not significant coefficients in the regression model.

The regression model was tested at fibrinogen levels ≥ 378 mg/dL (50^{th} percentile), representing elevated levels of fibrinogen. A significant regression was found ($F(10,6) = 7.290$, $p = .012$), with the model explaining 24% (Nagelkerke R^2) of the variance and correctly classifying 76% of the cases for all variables in the model. Individuals with stage 3 CKD and higher were 3.16 times more likely to exhibit CVD than those without CKD. Chronic Kidney Disease, $\beta = 1.15$, $t(15) = 3.16$, $p = .006$, was seen to be a significant coefficient in the regression model. A significant regression was found for serum cholesterol level, as well as for demographic variables including age and gender. Diabetes status and obesity were not significant coefficients in the regression model.

Comparison of the aforementioned models demonstrates that fibrinogen plays an effect modifying role on how CKD affects CVD as hypothesized and demonstrated in Table 15 and Figure 14. While in the elevated fibrinogen group the odds ratio was (3.16) much higher than 1 and statistically significant, the regular fibrinogen group had a non-significant low odds ratio at 1.95. The stark contrast in the odds ratio demonstrated the effect modifying role of fibrinogen. Additionally, different cofactors were significant in each fibrinogen level.

When the F/T ratio was tested for effect modification, the analysis was unsuccessful due to inadequate number of subjects. At the elevated level of F/T Ratio > 11.67, there were no subjects at every level of CKD. Therefore, comparison of odds ratio with the normal F/T ratio was impossible. Consequently the answer to this hypothesis was not determined by the data.

Hypothesis 2

Hypothesis 2 predicted that elevated inflammatory biomarkers modify the effect of CVD on CKD after controlling for known CKD risk factors like obesity, hypercholesterolemia, diabetes status, smoking status, and hypertension. The demographic variables of age and gender were also controlled for so that confounding factors would not influence the study results. Initially, a multiple regression analysis was performed to determine if elevated serum CKD stages predict cardiovascular health in individuals 20 years and older within the U.S. population after controlling for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and gender. Then, separate multiple regression analyses were run

for normal levels versus the elevated levels of hs-CRP in order to look for effect modification as demonstrated in Table 16 and Figure 15.

Table 16

Age- and Sex-Adjusted CKD ORs for CVD in Multivariate Regression Modeling controlling for different demographic and CVD risk factors.

	Total OR (95% CI) (N=23,320)	**CRP^- OR (95% CI) (N=6992)**	**CRP^+ OR (95% CI) (N=6838)**
Model 1 (CVD)	1.64 (1.40, 1.93)*	1.39 (1.05, 1.85)*	1.94 (1.47-2.58)**
Model 2 (CVD, obesity)	2.09 (1.75-2.49)**	1.67 (1.21, 2.30)*	2.17 (1.60, 2.95)**
Model 3 (CVD, obesity, diabetes status)	1.95 (1.62-2.35)**	1.57 (1.09, 2.27)*	1.94 (1.41,2.66)**
Model 4 (CVD, obesity, diabetes status, smoking status)	1.95 (1.61-2.35)**	1.60 (1.10-2.34)*	1.95 (1.42, 2.67)**
Model 5 (CVD, obesity, diabetes status, smoking status, hypercholesterolemia)	1.96 (1.55-2.47)**	1.66 (1.08-2.54)*	2.07 (1.60-2.68)**
Model 6 (CVD, obesity, diabetes status, smoking status, hypercholesterolemia, hypertension)	1.77 (1.41, 2.23)**	1.58 (1.04-2.41)*	1.81 (1.27-2.57)**

Note. *$p < .05$ **$p < .001$

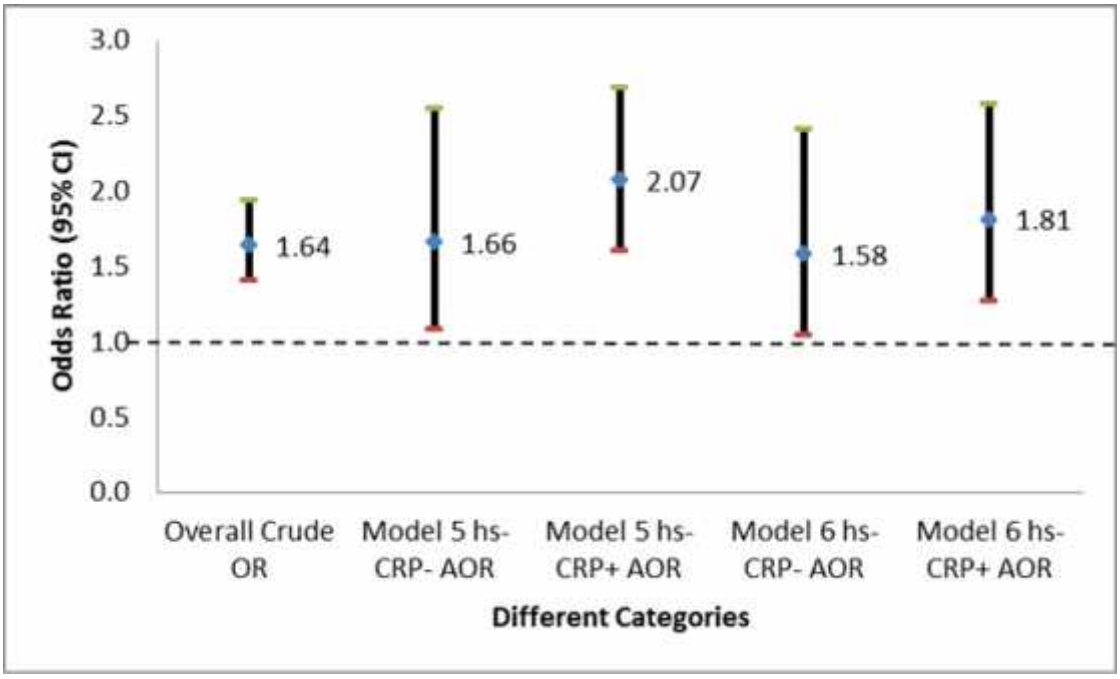

Figure 15. Crude and Adjusted Odds of CKD in individuals with CVD modified by hs-CRP level

The overall regression model was tested at hs-CRP levels. A significant regression was found ($F(10,67) = 161.730$, $p < .001$), with the model explaining 57% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with CVD were 1.96 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.67$, $t(76) = 5.79$, $p < .001$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity and diabetes status and for demographic variables including age and gender. Hypercholesterolemia and smoking status were found to non-significant factors in the logistic regression model.

The regression model was tested at hs-CRP levels less than 0.09 mg/dl (first quartile), representing baseline or low levels of hs-CRP. A significant regression was found ($F(10,66) = 38.816$, $p < .001$), with the model explaining 58% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with CVD were 1.66 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.50$, $t(75) = 2.34$, $p = .02$, was seen to be a statistically significant coefficient in the regression model. A significant regression was found for obesity and diabetes status and for demographic variables including age and gender. Hypercholesterolemia and smoking status were found to non-significant factors in the logistic regression model.

The regression model was tested at hs-CRP levels greater than 0.51 mg/dl (greater than third quartile), representing elevated levels of hs-CRP. A significant regression was found ($F(10,67) = 147.024$, $p < .001$), with the model explaining 57% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with CVD were 2.07times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.72$, $t(76) = 5.64$, $p < .001$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity and diabetes status and for demographic variables including age and gender. Hypercholesterolemia and smoking status were found to non-significant factors in the logistic regression model.

Comparison of the aforementioned models demonstrates that hs-CRP plays an effect modifying role on how CVD affects CKD as hypothesized as demonstrated in

Table 16 and Figure 15. While in the elevated hs-CRP group the odds ratio was (2.07) much higher than 1 and statistically significant, the regular hs-CRP group had a significant low odds ratio at 1.66. The stark contrast in the odds ratio demonstrated the effect modifying role of hs-CRP. Additionally, different cofactors were significant in each hs-CRP level.

Table 17

Age- and Sex-Adjusted CKD ORs for CVD in Multivariate Regression Modeling controlling for different demographic and CVD risk factors.

	Total OR (95% CI) (N=9167)	**Hcy^- OR (95% CI) (N=4609)**	**Hcy^+ OR (N=4558)**
Model 1 (CVD)	1.59 (1.13, 2.23)*	0.47 (0.24, 0.91)*	1.76 (1.26, 2.47)*
Model 2 (CVD, obesity)	2.17 (1.51, 3.10)*	0.54 (0.28, 1.06)	1.76 (1.25, 2.49)*
Model 3 (CVD, obesity, diabetes status)	2.02 (1.22, 3.35)*	0.64 (0.29, 1.43)	1.71 (1.11, 2.62)*
Model 4 (CVD, obesity, diabetes status, smoking status)	2.04 (1.22, 3.39)*	0.64 (0.27, 1.49)	1.88 (1.21, 2.90)*
Model 5 (CVD, obesity, diabetes status, smoking status, hypercholesterolemia)	2.14 (1.26, 3.66)*	0.56 (0.28, 1.16)	2.46 (1.39, 4.34)*
Model 6 (CVD, obesity, diabetes status, smoking status, hypercholesterolemia, hypertension)	2.03 (1.19, 3.48)*	0.39 (0.11, 1.33)	2.29 (1.44, 3.64)*

Note. *$p < .05$ **$p < .001$

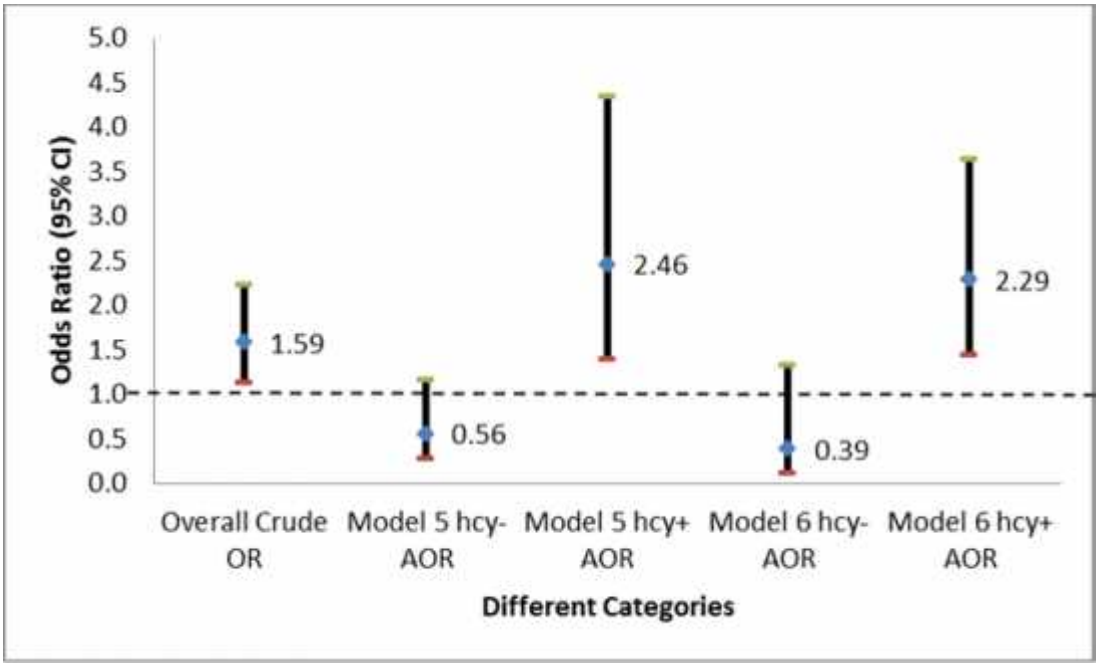

Figure 16. Crude and Adjusted Odds of CKD in individuals with CVD modified by hcy level

The regression models were tested at all homocysteine levels, normal homocysteine levels, and elevated homocysteine levels respectively to assess for effect modification as demonstrated in Table 17 and Figure 16. At all hcy levels, a significant regression was found ($F(10,21) = 63.036$, $p < .001$), with the model explaining 63% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with CVD and higher were 2.14 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.76$, $t(30) = 2.91$, $p < .05$, was seen to be a significant coefficient in the regression model. A significant regression was found for the obesity variable as well as for demographic variables including age and gender.

Hypercholesterolemia, diabetes status, and smoking status were found to be non-significant variables.

The regression model was tested at hcy levels less than 8.15 µmol/L (50^{th} percentile), representing baseline or low levels of hcy. A significant regression was found ($F(8,22) = 35.982$, $p < .001$), with the model explaining 56% (Nagelkerke R^2) of the variance and correctly classifying 97% of the cases for all variables in the model. Individuals with CVD were 0.56 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.57$, $t(29) = 1.63$, $p = 0.11$, was seen to be a statistically non-significant coefficient in the regression model. A significant regression was found for obesity and diabetes status and for demographic variables including age and gender. Hypercholesterolemia and smoking status were found to non-significant factors in the logistic regression model.

The regression model was tested at hcy levels ≥ 8.15 µmol/L (50^{th} percentile), representing elevated levels of hcy. A significant regression was found ($F(10,21) = 42.855$, $p < .001$), with the model explaining 64% (Nagelkerke R^2) of the variance and correctly classifying 91% of the cases for all variables in the model. Individuals with CVD and higher were 2.46 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.90$, $t(30) = 3.23$, $p = .003$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity and smoking status and for demographic variables including age and gender. Hypercholesterolemia and diabetes status were found to non-significant factors in the logistic regression model.

Comparison of the aforementioned models demonstrates that homocysteine plays an effect modifying role on how CVD affects CKD as hypothesized and as demonstrated in Table 17 and Figure 16. While in the elevated hcy group the odds ratio was (2.46) much higher than 1 and statistically significant, the regular hcy group had a non-significant low odds ratio at 0.56. The stark contrast in the odds ratio demonstrated the effect modifying role of hcy. Additionally, different cofactors were significant in each hcy level.

Table 18

Age- and Sex-Adjusted CKD ORs for CVD in Multivariate Regression Modeling controlling for different demographic and CVD risk factors.

	Total OR (95% CI) (N=2827)	**Fibrinogen$^-$ OR (95% CI) (N=1424)**	**(N=1403) Fibrinogen$^+$**
Model 1 (CVD)	1.78 (1.24, 2.57)*	1.78 (1.00, 3.16)	1.80 (1.20, 2.69)*
Model 2 (CVD, obesity)	2.59 (1.84, 3.66)*	1.98 (1.21, 3.25)*	2.17 (1.47, 4.26)*
Model 3 (CVD, obesity, diabetes status)	2.52 (1.80, 3.52)*	1.98 (1.22, 3.23)*	2.42 (1.41, 4.14)*
Model 4 (CVD, obesity, diabetes status, smoking status)	2.49 (1.73, 3.58)*	2.11 (1.27-3.51)*	2.56 (1.45, 4.54)*
Model 5 (CVD, obesity, diabetes status, smoking status, hypercholesterolemia)	2.87 (1.71, 4.82)*	2.35 (0.59, 9.41)	2.96 (1.58, 5.54)*
Model 6 (CVD, obesity, diabetes status, smoking status, hypercholesterolemia, hypertension)	2.78 (1.61, 4.79)*	2.35 (0.58, 9.47)	2.92 (1.52, 5.60)*

Note. *$p < .05$ **$p < .001$

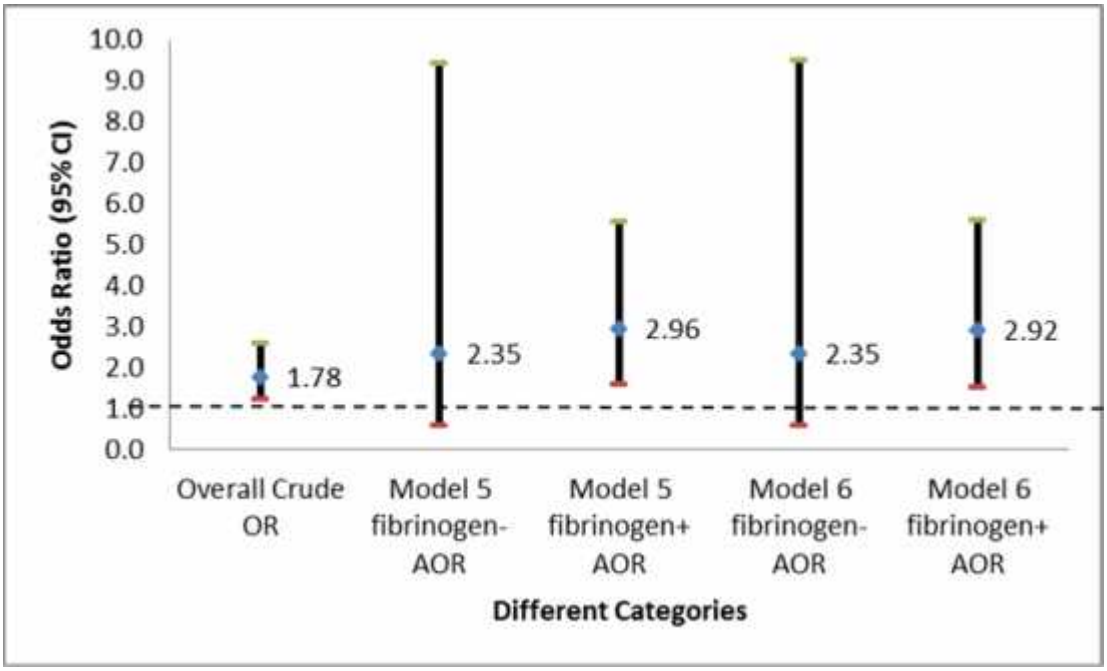

Figure 17. Crude and Adjusted Odds of CKD in individuals with CVD modified by fibrinogen level

The regression models were tested at all fibrinogen levels, normal fibrinogen levels, and elevated fibrinogen levels respectively to assess for effect modification as demonstrated in Table 18 and Figure 17. At all fibrinogen levels, a significant regression was found ($F(10,6) = 16.413$, $p = .001$), with the model explaining 52% (Nagelkerke R^2) of the variance and correctly classifying 91% of the cases for all variables in the model. Individuals with CVD were 2.87 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 1.05$, $t(15) = 4.34$, $p = .001$, was seen to be a significant coefficient in the regression model. A significant regression was found for obesity as well as for demographic variables including age and gender. Hypercholesterolemia, diabetes status, and smoking status were found to be non-significant factors in the regression equation.

The overall regression model was tested at fibrinogen levels less than 378 mg/dL (50^{th} percentile), representing baseline or low levels of fibrinogen. A significant regression was found ($F(8,8) = 3.472$, $p < .05$), with the model explaining 54% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with CVD were 2.35 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 0.85$, $t(15) = 1.31$, $p = .21$, was seen to be a statistically non-significant coefficient in the regression model. A significant regression was found for obesity as well as for demographic variables including age and gender. Hypercholesterolemia, diabetes status, and smoking status were found to be non-significant factors in the regression equation.

The regression model was tested at fibrinogen levels ≥ 378 mg/dL (50^{th} percentile), representing elevated levels of fibrinogen. A significant regression was found ($F(8,8) = 6.722$, $p < .05$), with the model explaining 47% (Nagelkerke R^2) of the variance and correctly classifying 86% of the cases for all variables in the model. Individuals with CVD were 2.96 times more likely to exhibit CKD than those without CVD. CVD, $\beta = 1.09$, $t(15) = 3.70$, $p = .002$, was seen to be a significant coefficient in the regression model. A significant regression was found for age. Hypercholesterolemia, diabetes status, gender, diabetes status, and smoking status were found to be non-significant factors in the regression equation.

Comparison of the aforementioned models demonstrates that fibrinogen plays an effect modifying role on how CKD affects CVD as hypothesized. While in the elevated fibrinogen group the odds ratio was (2.96) much higher than 1 and statistically

significant, the regular fibrinogen group had a non-significant low odds ratio at 2.35. The stark contrast in the odds ratio demonstrated the effect modifying role of fibrinogen. Additionally, different cofactors were significant in each fibrinogen level.

When the F/T ratio was tested for effect modification, the analysis was unsuccessful due to inadequate number of subjects as demonstrated in Table 17 and Figure 16. At the elevated level of F/T (Ratio>11.67), there were no subjects at every level of CVD. Therefore, comparison of odds ratio with the normal F/T ratio was impossible. Consequently the answer to this hypothesis was not determined by the data.

Hypothesis 3

Hypothesis 3 predicted that elevated inflammatory biomarkers have an additive effect on CRS along with CVD risk factors like obesity, hypercholesterolemia, diabetes status, smoking status, and hypertension. The demographic variables of age and gender were also factors that were being tested. Initially, a multiple regression analysis was performed to determine if elevated serum hs-CRP predict cardiovascular health in individuals 20 years and older within the U.S. population in addition to obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic variables including age and gender. Then, separate multiple regression analyses were run for homocysteine, fibrinogen, and F/T ratio levels respectively in order to study the additive effect.

Table 19

Regression Analysis Predicting Cardiorenal Syndrome From Elevated hs-CRP Levels of Individuals Aged 20 Years and Older, NHANES, 1999-2010

Variables	β	Adjusted OR	95% Confidence Interval	p-value
Elevated Serum hs-CRP	0.46	1.58	1.18-2.12	0.003
Medical Variables				
Obese BMI (≥30)	0.42	1.53	1.08-2.16	0.02
Presence of Diabetes	1.35	3.87	2.85-5.27	<0.001
	0.33	1.40	1.05-1.86	0.02
Hypercholesterolemia				
Current Smoker (Reference=Never Smoker)	0.81	2.26	1.38-3.68	0.001
Demographic Variables				
Age	0.16	1.17	1.15-1.20	<0.001
Gender (Reference=Female)	-0.20	0.82	0.62-1.09	0.171

Note. $F(8,69) = 54.164$, $p < .001$ $R^2 = .492$ ($p < .001$)

As demonstrated in Table 19, the regression model was tested for hs-CRP as an additive effect along with obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. A significant regression was found ($F(10,66) = 43.732$, $p < .001$), with the model explaining 52% (Nagelkerke R^2) of the variance and correctly classifying 96% of the cases for all variables in the model. Individuals with elevated hs-CRP were 1.58 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.82$, $t(75) = 2.88$, $p < .05$, was seen to be a significant coefficient in the regression model. Additionally, age, smoking status, obesity, cholesterol status, and diabetes status were

found to be significant coefficients in the regression model. However, gender was found to not be significant coefficients of the regression model.

Table 20

Regression Analysis Predicting Cardiorenal Syndrome From Elevated hcy Levels of Individuals Aged 20 Years and Older, NHANES, 1999-2010

Variables	β	Adjusted OR	95% Confidence Interval	p-value
Elevated Serum hcy	1.21	3.35	2.02-5.56	<0.001
Medical Variables				
Obese BMI (≥30)	0.12	1.13	0.76-1.68	0.54
Presence of Diabetes	1.37	3.93	2.12-7.27	<0.001
	0.18	1.19	0.75-1.90	0.44
Hypercholesterolemia				
Current Smoker (Reference=Never Smoker)	0.32	1.37	0.71-2.66	0.34
Demographic Variables				
Age	0.16	1.17	1.14-1.21	<0.001
Gender (Reference=Female)	-0.67	0.94	0.61-1.44	0.754

Note. $F(8,23) = 40.550$, $p < .001$ $R^2 = .543$ ($p < .001$)

As demonstrated in Table 20, the regression model was tested for homocysteine as an additive effect along with obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. A significant regression was found ($F(8,23) = 40.550$, $p < .001$), with the model explaining 54% (Nagelkerke R^2) of the variance and correctly classifying 97% of the cases for all variables in the model. Individuals with elevated hcy were 3.35 times more likely to exhibit CRS than those without CRS. Elevated hcy, $\beta = 1.21$, $t(30) = 4.86$, $p < 0.001$, was seen to be a significant coefficient in the regression model. Additionally, age and diabetes status were found to be significant coefficients in

the regression model. However, obesity, smoking status, cholesterol status, and gender were found to not be significant coefficients of the regression model.

Table 21

Regression Analysis Predicting Cardiorenal Syndrome From Elevated fibrinogen Levels of Individuals Aged 20 Years and Older, NHANES, 1999-2010

Variables	β	Adjusted OR	95% Confidence Interval	p-value
Elevated Serum fibrinogen	0.36	1.58	0.05-2.12	0.827
Medical Variables				
Obese BMI (≥30)	0.30	1.35	0.14-13.08	0.782
Presence of Diabetes	--	--	--	--
	2.38	10.80	0.45-259.88	0.132
Hypercholesterolemia				
Current Smoker (Reference=Never Smoker)	--	--	--	--
Demographic Variables				
Age	0.28	1.32	1.01-1.75	0.04
Gender (Reference=Female)	-0.80	0.45	0.04-5.52	0.507

Note. $F(5,11) = 8.593$, $p = 0.002$ $R^2 = .332$ ($p < .001$)

As demonstrated in Table 21, the regression model was tested for fibrinogen as an additive effect along with obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Upon running the regression including diabetes status and smoking status was causing quasi-complete separation in the data. Therefore, fibrinogen, obesity, age, hypercholesterolemia, and gender were the only variables included. A significant regression was found ($F(5,11) = 8.593$, $p = 0.002$), with the model explaining 33% (Nagelkerke R^2) of the variance and correctly classifying 99% of the cases for all

variables in the model. Individuals with elevated fibrinogen were 1.43 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, β = 0.36, t (15) = 0.22, p = 0.83, was seen to be a non-significant coefficient in the regression model. Age was the only variable that was significant in the model. None of the other factors were statistically significant coefficients in the regression model.

Table 22

Regression Analysis Predicting Cardiorenal Syndrome From Elevated F/T Levels of Individuals Aged 20 Years and Older, NHANES, 1999-2010

Variables	β	Adjusted OR	95% Confidence Interval	p-value
Elevated Serum F/T	0.27	1.31	0.23-7.57	0.762
Medical Variables				
Obese BMI (≥30)	0.41	1.50	0.32-7.19	0.604
Presence of Diabetes	1.69	10.48	1.17-25.23	0.031
	0.57	1.06	0.16-6.95	0.952
Hypercholesterolemia				
Current Smoker (Reference=Never Smoker)	0.48	1.61	0.20-13.07	0.650
Demographic Variables				
Age	0.22	1.25	1.04-1.49	0.017
Gender (Reference=Female)	--	--	--	--

Note. F(7,58) = 13.443, $p < .001$ $R^2 = .154$ ($p < .001$)

As demonstrated in Table 22, the regression model was tested for ferritin/transferrin ratio as an additive effect along with obesity, age, hypercholesterolemia, diabetes status, and smoking status. Gender was excluded because only females had ferritin levels taken. Therefore, F/T, obesity, age, hypercholesterolemia, diabetes status, and smoking status were the only variables

included. A significant regression was found ($F(7,58) = 13.443$, $p < 0.001$), with the model explaining 15% (Nagelkerke R^2) of the variance and correctly classifying 99% of the cases for all variables in the model. Individuals with elevated F/T were 1.31 times more likely to exhibit CRS than those without CRS. Elevated F/T, $\beta = 0.27$, $t(64) = 0.30$, $p = 0.77$, was seen to be a non-significant coefficient in the regression model. Age and diabetes status were the only variable that were significant in the model. None of the other factors (hypercholesterolemia, smoking status, and obesity) were statistically significant coefficients in the regression model.

Hypothesis 4

Hypothesis 4 predicted that demographic factors like race/ethnicity, family income, or education level modify the effect of inflammatory biomarkers on CRS after controlling for known CVD risk factors like obesity, hypercholesterolemia, diabetes status, and smoking status. The demographic variables of age and gender were also controlled for so that confounding factors would not influence the study results.

The effect modification of main demographic factors like race/ethnicity, family income, and education level were systematically tested in nine stepwise logistic regression models for hs-CRP, homocysteine, and fibrinogen respectively. The hs-CRP was categorized by comparing less than 1 mg/L versus greater than 2 mg/L. Initially, a multiple regression analysis was performed to determine if among the Hispanic subpopulation, elevated serum hs-CRP predicts cardiovascular health in individuals 20 years and older within the U.S. population after controlling for obesity, hypercholesterolemia, diabetes status, smoking status, as well as for demographic

variables including age and gender. Then, separate multiple regression analyses were run for hs-CRP levels in Non-Hispanic White and Non-Hispanic Black subpopulations as well in order to assess for effect modification.

Likewise, six stepwise multiple regression models were reported, assessing for effect modification with regards to family income and education level. Three stepwise logistic regression models were run for the total sample, below 200% poverty level, and ≥ 200% poverty level. Finally, three stepwise logistic regression models were run for different education levels—less than high school graduate, high school graduate, and at least some college education.

Table 23

Age- and Sex-Adjusted hs-CRP ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by ethnicity.

	Hispanic OR (95% CI) (N=3,146)	**Non-Hispanic White OR (95% CI) (N=6,340)**	**Non-Hispanic Black OR (95% CI) (N=2,345)**
Model 1 (hs-CRP)	3.24 (1.59, 6.57)*	2.34 (1.46, 3.74)*	2.96 (1.82-4.81)**
Model 2 (hs-CRP, obesity)	2.81 (1.38-5.72)*	2.01 (1.21-3.33)*	2.86 (1.67-4.88)**
Model 3 (hs-CRP, obesity, diabetes status)	3.43 (1.54-7.63)*	2.03 (1.16-3.53)*	3.02 (1.70-5.39)*
Model 4 (hs-CRP, obesity, diabetes status, smoking status)	3.59 (1.62-7.94)*	1.92 (1.10-3.38)	3.00 (1.71, 5.28)*
Model 5 (hs-CRP, obesity, diabetes status, smoking status, hypercholesterolemia)	4.31 (1.76-10.52)*	2.26 (1.29-3.98)*	2.88 (1.59-5.23)*

Note. *$p < .05$ **$p < .001$

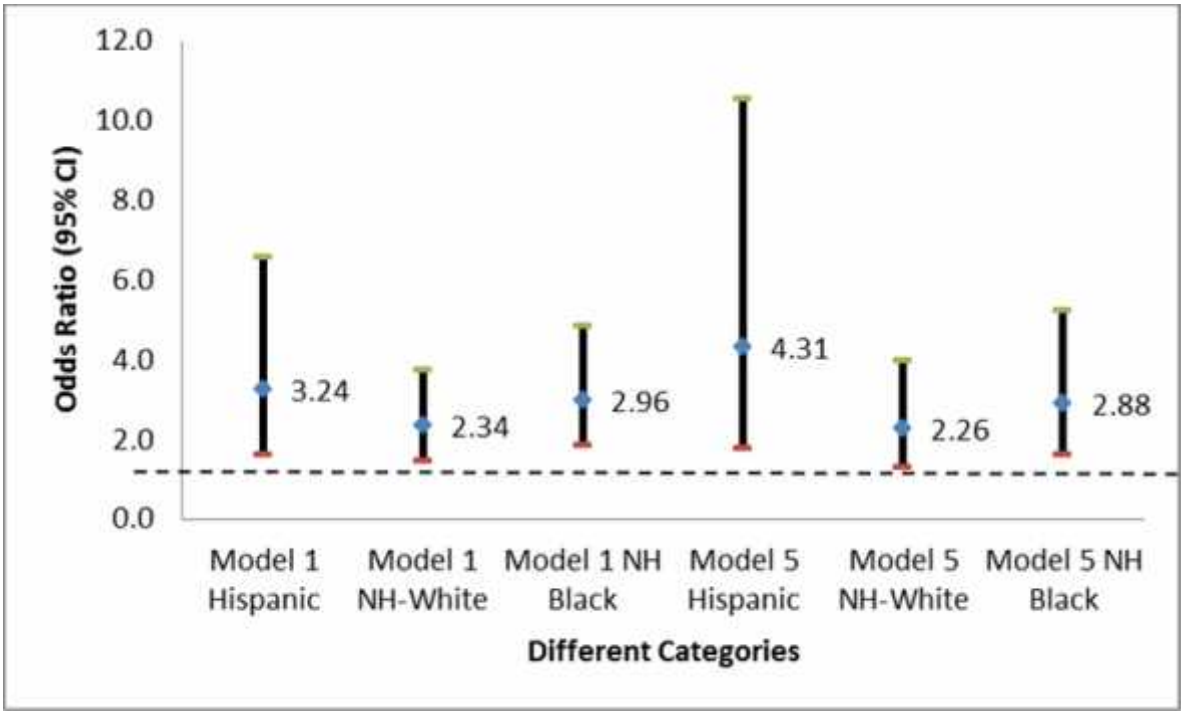

Figure 18. Effect of Race on the relationship between hs-CRP and CRS before and after controlling for CRS risk factors

Initially, the total sample was stratified to assess for effect modification by race as seen in Table 23 and Figure 18. The first group of regression models was calculated for Hispanic individuals. A significant regression was found ($F(10,60) = 12.922$, $p < .001$), with the model explaining 30% (Nagelkerke R^2) of the variance and correctly classifying 98% of the cases for all variables in the model. Individuals with elevated hs-CRP were 4.31 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 1.46$, $t(69) = 3.26$, $p < .05$, was seen to be a non-significant coefficient in the regression model. Additionally, age, diabetes status, and obesity were found to be the only significant coefficients in the regression model. However, smoking status, gender, and cholesterol status were found to not be significant coefficients of the regression model.

The regression model was tested for Non-Hispanic Whites. A significant regression was found ($F(10,66) = 43.732$, $p < .001$), with the model explaining 52% (Nagelkerke R^2) of the variance and correctly classifying 96% of the cases for all variables in the model. Individuals with elevated hs-CRP were 2.26 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.82$, $t(75) = 2.88$, $p < .05$, was seen to be a significant coefficient in the regression model. Additionally, age, smoking status, obesity, cholesterol status, and diabetes status were found to be significant coefficients in the regression model. However, gender was found to not be significant coefficients of the regression model.

The regression model was tested for Non-Hispanic Blacks. A significant regression was found ($F(10,52) = 31.408$, $p < .001$), with the model explaining 37% (Nagelkerke R^2) of the variance and correctly classifying 95% of the cases for all variables in the model. Individuals with elevated hs-CRP were 2.88 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 1.06$, $t(61) = 3.54$, $p < 0.001$, was seen to be a significant coefficient in the regression model. Additionally, age, smoking status, diabetes status, and cholesterol status were found to be significant coefficients in the regression model. However, obesity and gender were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that ethnicity plays an effect modifying role on how hs-CRP levels affect CRS as hypothesized. While in the Non-Hispanic White, Non-Hispanic Black, and Hispanic populations the odds ratio of CRS was much higher than 1 and statistically significant, the Non-Hispanic White

population had the lowest odds ratio at 2.26 as seen in Table 23 and Figure 18. The Hispanic population had the highest odds ratio at 4.31 when controlling for other CVD risk factors. Additionally, different cofactors were significant in each ethnic subpopulation.

Table 24

Age- and Sex-Adjusted hs-CRP ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by income level.

	Total OR (95% CI) (N=20,850)	**Below 200% Poverty (95% CI) (N=9249)**	**≥ 200% Poverty (95% CI) (N=11601)**
Model 1 (hs-CRP)	2.33 (1.62-3.37)*	2.08 (1.31-3.32)*	2.23 (1.25-3.98)*
Model 2 (hs-CRP, obesity)	2.05 (1.38-3.03)*	1.72 (1.06-2.81)*	2.08 (1.13-3.83)*
Model 3 (hs-CRP, obesity, diabetes status)	2.18 (1.42-3.34)*	1.94 (1.11-3.41)*	2.09 (1.12-3.91)*
Model 4 (hs-CRP, obesity, diabetes status, smoking status)	2.08 (1.35-3.19)*	1.85 (1.05-3.25)*	2.06 (1.10-3.85)*
Model 5 (hs-CRP, obesity, diabetes status, smoking status, hypercholesterolemia)	2.17 (1.73-3.43)**	1.74 (0.88-3.42)	2.33(1.21-4.52)*

Note. *$p < .05$ **$p < .001$

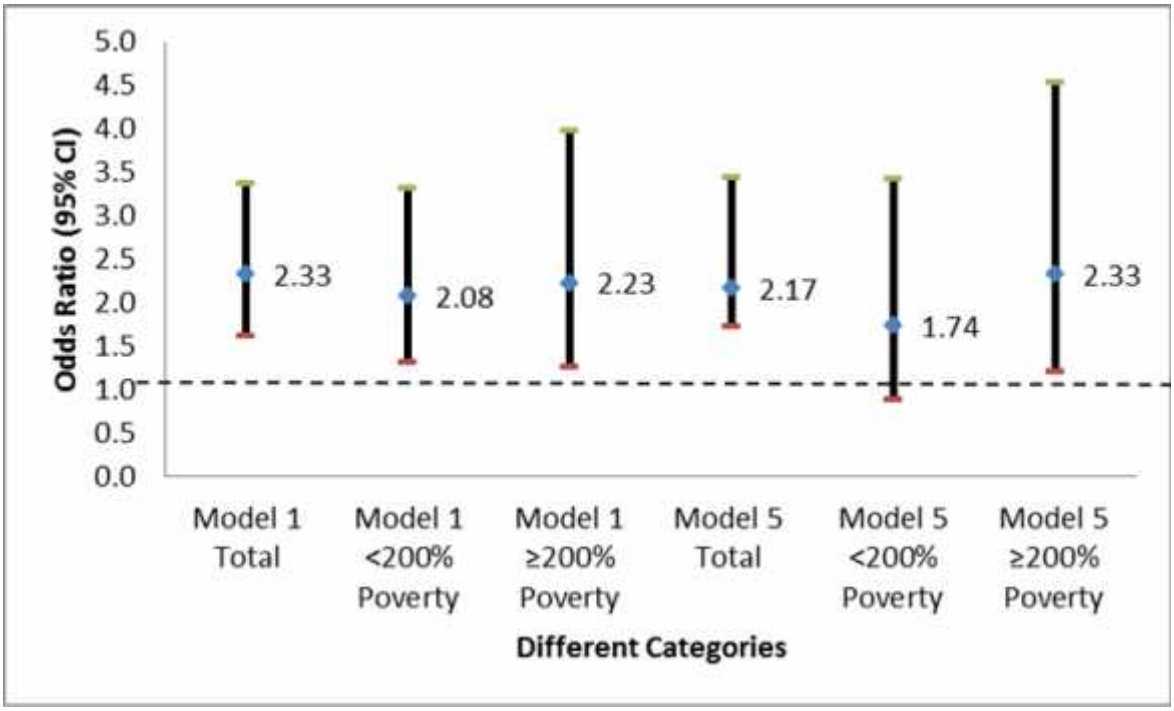

Figure 19. Effect of Income Level on the relationship between hs-CRP and CRS before and after controlling for CRS risk factors

Initially, the overall regression model was tested to determine how elevated hs-CRP played a role on the total sample as seen in Table 24 and Figure 19. A significant regression was found ($F(10,67) = 46.344$, $p < .001$), with the model explaining 47% (Nagelkerke R^2) of the variance and correctly classifying 96% of the cases for all variables in the model. Individuals with elevated hs-CRP were 2.17 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.77$, $t(76) = 3.36$, $p < .001$, was seen to be a significant coefficient in the regression model. Additionally, obesity, age, smoking status, and cholesterol status were found to be significant coefficients of the regression model. Gender was the only variable found to be not significant in the model.

The total sample was stratified to assess for effect modification. The regression model was tested at less than 200% of federal poverty levels, representing individuals in a low socioeconomic status. A significant regression was found ($F(10,66) = 25.518$, $p < .001$), with the model explaining 46% (Nagelkerke R^2) of the variance and correctly classifying 93% of the cases for all variables in the model. Individuals with elevated hs-CRP were 1.73 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.55$, $t(75) = 1.63$, $p=0.11$, was seen to be a non-significant coefficient in the regression model. Additionally, age, obesity, cholesterol status, and diabetes status was found to be significant coefficients in the regression model. However, smoking status and gender were found to not be significant coefficients of the regression model.

The overall regression model was tested at ≥ 200% of federal poverty levels, representing normal to high socioeconomic status. A significant regression was found ($F(10,67) = 29.908$, $p < .001$), with the model explaining 48% (Nagelkerke R^2) of the variance and correctly classifying 97% of the cases for all variables in the model. Individuals with elevated hs-CRP were 2.33 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.85$, $t(76) = 2.55$, $p < .05$, was seen to be a significant coefficient in the regression model. Additionally, obesity, age, cholesterol status, and diabetes status was found to be significant coefficients in the regression model. However, gender and smoking status were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that income level does not play an effect modifying role on how hs-CRP levels affect CRS as hypothesized.

While in all three categories, gender was not a significant variable, when stratified by socioeconomic status, smoking status was not significant as well as seen in Table 24 and Figure 19. In every model in the low SES category, the odds ratio and confidence intervals of elevated hs-CRP on CRS was lower in individuals at the high income level.

Table 25

Age- and Sex-Adjusted CRP ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by education level.

	Less than High School Graduate OR (95% CI) (N=5,820)	**High School Graduate (95% CI) (N=4,632)**	**At least Some College (95% CI) (N=11,129)**
Model 1 (hs-CRP)	2.93 (1.79-4.79)**	2.97 (1.57-5.62)*	1.89 (0.91-3.94)
Model 2 (hs-CRP, obesity)	2.26 (1.31-3.89)*	2.65 (1.33-5.30)*	1.89 (0.86-4.15)
Model 3 (hs-CRP, obesity, diabetes status)	2.80 (1.52-5.15)*	2.47 (1.16-5.26)	2.03 (0.87-4.76)
Model 4 (hs-CRP, obesity, diabetes status, smoking status)	2.67 (1.47-4.84)*	2.48 (1.16-5.30)	1.89 (0.81-4.43)
Model 5 (hs-CRP, obesity, diabetes status, smoking status, hypercholesterolemia)	2.55 (1.25-5.19)*	3.70 (1.59-8.61)*	1.75 (0.83-3.69)

Note. *$p < .05$ **$p < .001$

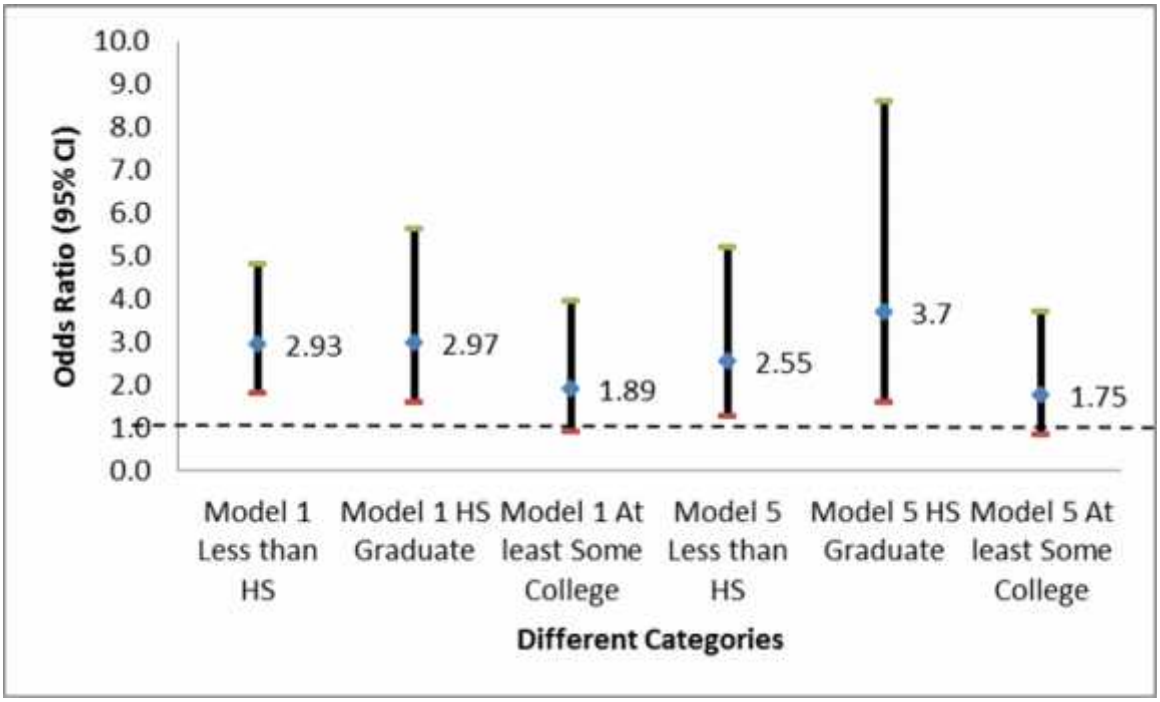

Figure 20: Effect of Education Level on the relationship between hs-CRP and CRS before and after controlling for CRS risk factors

The total sample was stratified by education level to assess for effect modification as seen in Table 25 and Figure 20. Initially, the overall regression model was tested to determine how elevated hs-CRP played a role on individuals with less than a high school education. A significant regression was found ($F(10,67) = 15.899$, $p < .001$), with the model explaining 41% (Nagelkerke R^2) of the variance and correctly classifying 92% of the cases for all variables in the model. Individuals with elevated hs-CRP were 2.55 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.94$, $t(76) = 2.62$, $p < 0.05$, was seen to be a non-significant coefficient in the regression model. Out of the other variables, age, obesity, cholesterol status, and diabetes status were found

to be the only significant coefficient in the regression model. However, smoking status and gender were found to not be significant coefficients of the regression model.

The regression model was tested for high school graduates, representing individuals who have graduated high school or who have at least acquired a GED. A significant regression was found ($F(10,67) = 22.730$, $p < .001$), with the model explaining 51% (Nagelkerke R^2) of the variance and correctly classifying 96% of the cases for all variables in the model. Individuals with elevated hs-CRP were 3.70 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 1.31$, $t(76) = 3.08$, $p = .003$, was seen to be a significant coefficient in the regression model. Additionally, age, cholesterol status, smoking status, and diabetes status were found to be significant coefficients in the regression model. However, gender and obesity were found to not be significant coefficients of the regression model.

Finally, the overall regression was tested for individuals with at least some college education, representing the educated stratum of society. A significant regression was found ($F(10,67) = 26.178$, $p < .001$), with the model explaining 47% (Nagelkerke R^2) of the variance and correctly classifying 98% of the cases for all variables in the model. Individuals with elevated hs-CRP were 1.75 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = 0.56$, $t(76) = 1.49$, $p = 0.14$, was seen to be a non-significant coefficient in the regression model. Additionally, age, obesity, cholesterol status, and diabetes status were found to be significant coefficients in the regression model. However, smoking status and gender were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that education level plays an effect modifying role on how hs-CRP levels affect CRS as hypothesized. When comparing individuals with at most a high school education with individuals with more than a high school education, individuals with more than a high school education have an odds ratio close to 1 as seen in Table 25 and Figure 20. Additionally, in every model tested in the high school graduate category, the odds ratio of elevated hs-CRP on CRS was higher in individuals with only a high school education. The covariates that were significant were different in each model.

Table 26

Age- and Sex-Adjusted Hcy ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by ethnicity.

	Hispanic OR (95% CI) (N=1,944)	**Non-Hispanic White OR (95% CI) (N=3,986)**	**Non-Hispanic Black OR (95% CI) (N=1,688)**
Model 1 (Hcy)	1.06 (0.46-2.46)	3.19 (1.93, 5.28)**	2.68 (1.28-5.64)*
Model 2 (Hcy, obesity)	1.10 (0.50-2.42)	3.46 (2.01-5.95)**	2.64 (1.22-5.71)*
Model 3 (Hcy, obesity, diabetes status)	0.44 (0.13-1.46)	4.91 (3.16-7.62)**	3.36 (1.23-9.18)*
Model 4 (Hcy, obesity, diabetes status, smoking status)	0.44 (0.12-1.56)	5.00 (3.07-8.16)**	3.10 (1.15-8.38)*
Model 5 (Hcy, obesity, diabetes status, smoking status, hypercholesterolemia)	0.49 (0.15-1.67)	4.15 (2.24-7.66)**	2.59 (0.91-7.39)

Note. *$p < .05$ **$p < .001$

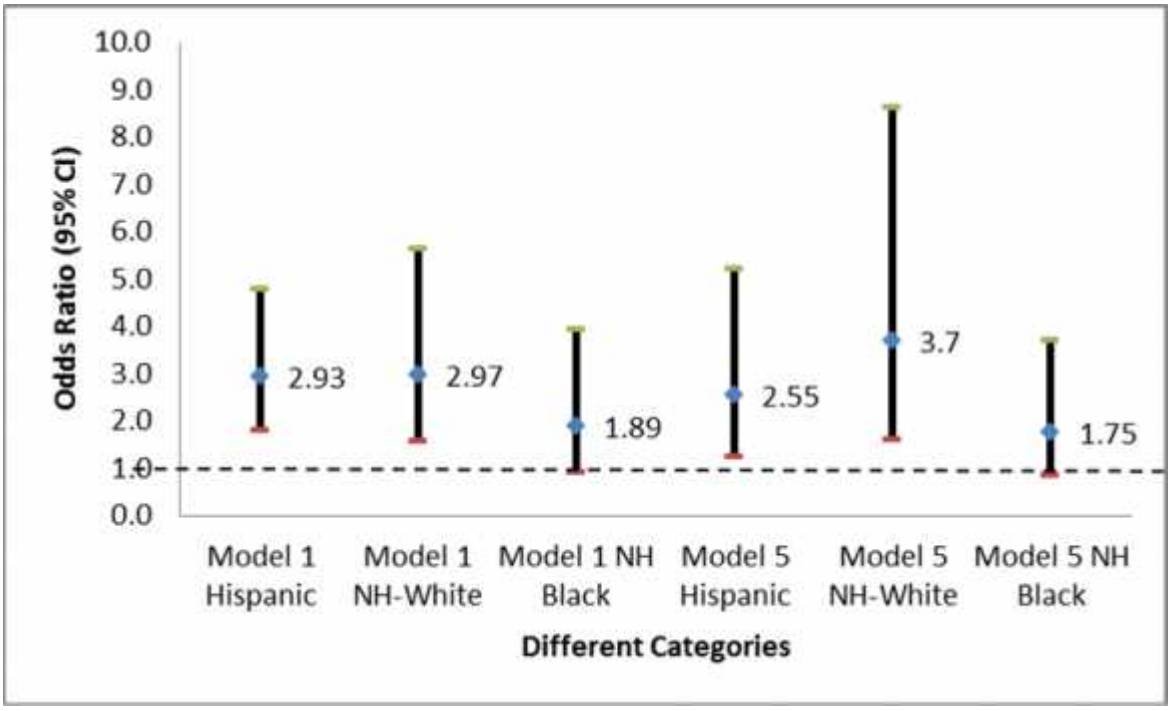

Figure 21: Effect of Race on the relationship between homocysteine and CRS before and after controlling for CRS risk factors

Initially, the total sample was stratified to assess for effect modification by race as seen in Table 26 and Figure 21. The first group of regression models was calculated for Hispanic individuals. A significant regression was found ($F(10,11) = 42.520$, $p < .001$), with the model explaining 18% (Nagelkerke R^2) of the variance and correctly classifying 98% of the cases for all variables in the model. Individuals with elevated homocysteine were 0.49 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = -0.71$, $t(20) = -1.21$, $p > .05$, was seen to be a non-significant coefficient in the regression model. Additionally, age and diabetes status were found to be the only significant

coefficients in the regression model. However, obesity, smoking status, gender, and cholesterol status were found to not be significant coefficients of the regression model.

The regression model was tested for Non-Hispanic Whites. A significant regression was found ($F(10,19) = 49.480$, $p < .001$), with the model explaining 60% (Nagelkerke R^2) of the variance and correctly classifying 97% of the cases for all variables in the model. Individuals with elevated homocysteine were 4.15 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 1.42$, $t(28) = 4.74$, $p < .001$, was seen to be a significant coefficient in the regression model. Additionally, age and diabetes status were found to be significant coefficients in the regression model. However, smoking status, obesity, cholesterol status, and gender were found to not be significant coefficients of the regression model.

The regression model was tested for Non-Hispanic Blacks. A significant regression was found ($F(10,12) = 34.348$, $p < .001$), with the model explaining 45% (Nagelkerke R^2) of the variance and correctly classifying 96% of the cases for all variables in the model. Individuals with elevated homocysteine were 2.59 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 0.95$, $t(21) = 1.89$, $p = 0.07$, was seen to be a non-significant coefficient in the regression model. Additionally, age, smoking status, and cholesterol status were found to be significant coefficients in the regression model. However, obesity, gender, and diabetes status were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that ethnicity play an effect modifying role on how homocysteine levels affect CRS as hypothesized. While in

the Non-Hispanic White and Non-Hispanic Black the odds ratio of CRS were much higher than 1, in the Hispanic population the odds ratio was close to 1 as seen in Table 26 and Figure 21. Additionally, different cofactors were significant (i.e. age, smoking status and cholesterol status) in the Non-Hispanic Black population than the Non-Hispanic White and Hispanic population (i.e. age and diabetes status). In this analysis, homocysteine did not play a significant role in the Hispanic population.

Table 27

Age- and Sex-Adjusted Hcy ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by income level.

	Total OR (95% CI) (N=7,567)	**Below 200% Poverty (95% CI) (N=3212)**	**≥ 200% Poverty (95% CI) (N=4355)**
Model 1 (Hcy)	2.92 (1.94-4.38)*	2.92 (1.76-4.83)**	2.80 (1.48-5.32)*
Model 2 (Hcy, obesity)	3.06 (2.00-4.70)**	3.15 (1.91-5.21)**	2.82 (1.48-5.39)*
Model 3 (Hcy, obesity, diabetes status)	3.50 (2.40-5.10)**	4.29 (2.10-8.75)**	3.08 (1.47-6.42)*
Model 4 (Hcy, obesity, diabetes status, smoking status)	3.51 (2.35-5.24)**	4.27 (2.19-8.33)**	3.19 (1.45-7.03)*
Model 5 (Hcy, obesity, diabetes status, smoking status, hypercholesterolemia)	3.13 (1.89-5.18)**	3.16 (1.77-5.64)**	3.00 (1.27-7.12)*

Note. *$p < .05$ **$p < .001$

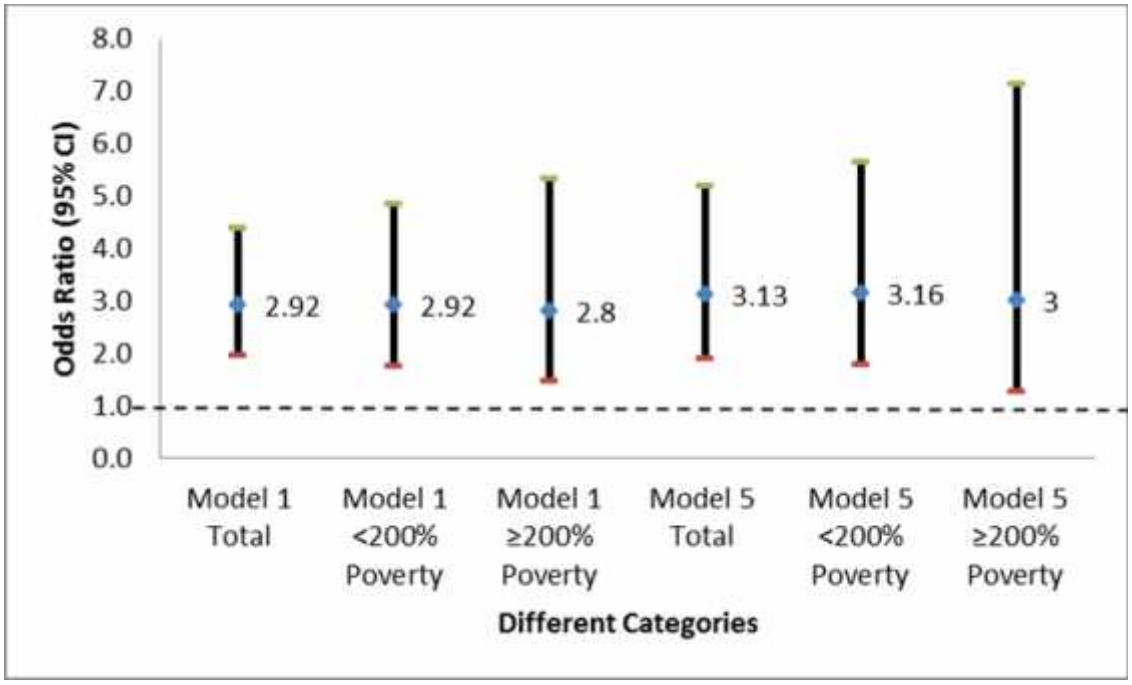

Figure 22: Effect of Income Level on the relationship between Homocysteine and CRS before and after controlling for CRS risk factors

Initially, the overall regression model was tested to determine how elevated homocysteine played a role on the total sample as seen in Table 27 and Figure 22. A significant regression was found ($F(10,21) = 52.555$, $p < .001$), with the model explaining 53% (Nagelkerke R^2) of the variance and correctly classifying 97% of the cases for all variables in the model. Individuals with elevated homocysteine were 3.13 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 1.14$, $t(30) = 4.63$, $p < .001$, was seen to be a significant coefficient in the regression model. Additionally, age and diabetes status were found to be the only significant coefficients in the regression

model. However, obesity, gender, smoking status, and cholesterol status were found to not be significant coefficients of the regression model.

The total sample was stratified to assess for effect modification. The regression model was tested at less than 200% of federal poverty levels, representing individuals in a low socioeconomic status. A significant regression was found ($F(10,15) = 17.752$, $p < .001$), with the model explaining 56% (Nagelkerke R^2) of the variance and correctly classifying 95% of the cases for all variables in the model. Individuals with elevated homocysteine were 3.16 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 1.15$, $t(24) = 4.10$, $p < .001$, was seen to be a significant coefficient in the regression model. Additionally, age, obesity, cholesterol status, and diabetes status was found to be significant coefficients in the regression model. However, smoking status and gender were found to not be significant coefficients of the regression model.

The overall regression model was tested at ≥ 200% of federal poverty levels, representing normal to high socioeconomic status. A significant regression was found ($F(10,21) = 31.794$, $p < .001$), with the model explaining 52% (Nagelkerke R^2) of the variance and correctly classifying 98% of the cases for all variables in the model. Individuals with elevated homocysteine were 3.00 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 1.10$, $t(30) = 2.60$, $p < .05$, was seen to be a significant coefficient in the regression model. Additionally, age and diabetes status was found to be significant coefficients in the regression model. However, obesity, smoking status, and cholesterol status were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that income level plays an effect modifying role on how homocysteine levels affect CRS as hypothesized. While in the overall model and ≥ 200% of federal poverty levels only diabetes status and age played a role in affecting CRS, in the lower socioeconomic status group only, cholesterol status and obesity played a role as seen in Table 27 and Figure 22. Additionally, in every model in the low SES category, the odds ratio and confidence intervals of elevated homocysteine on CRS were higher in individuals at the low income level.

Table 28

Age- and Sex-Adjusted Hcy ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by education level.

	Less than High School Graduate OR (95% CI) (N=2,117)	**High School Graduate (95% CI) (N=1,918)**	**At least Some College (95% CI) (N=3,905)**
Model 1 (Hcy)	2.00 (1.11-3.61)*	3.99 (1.77-9.02)*	2.87 (1.39-5.92)*
Model 2 (Hcy, obesity)	2.11 (1.16-3.84)*	4.10 (1.76-9.52)*	3.02 (1.38-6.59)*
Model 3 (Hcy, obesity, diabetes status)	1.45 (0.64-3.31)	10.46 (1.76-62.21)*	4.31 (1.87-9.93)*
Model 4 (Hcy, obesity, diabetes status, smoking status)	1.53 (0.62-3.79)	9.75 (1.64-57.94)*	4.25 (1.78-10.17)*
Model 5 (Hcy, obesity, diabetes status, smoking status, hypercholesterolemia)	1.42 (0.62-3.23)	7.12 (1.45-35.06)*	3.83 (1.58-9.28)*

Note. *$p < .05$ **$p < .001$

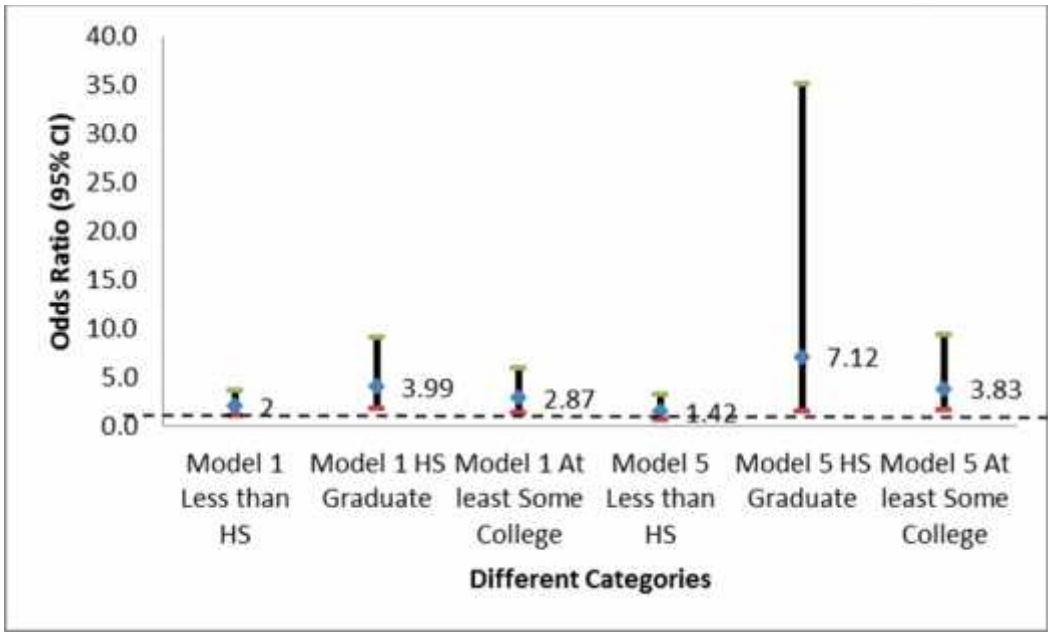

Figure 23: Effect of education level on the relationship between Homocysteine and CRS before and after controlling for CRS risk factors

The total sample was stratified by education level to assess for effect modification as seen in Table 28 and Figure 23. Initially, the overall regression model was tested to determine how elevated homocysteine played a role on individuals with less than a high school education. A significant regression was found ($F(10,12) = 14.682$, $p < .001$), with the model explaining 55% (Nagelkerke R^2) of the variance and correctly classifying 92% of the cases for all variables in the model. Individuals with elevated homocysteine were 1.42 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 0.35$, $t(21) = 0.88$, $p = 0.39$, was seen to be a non-significant coefficient in the regression model. Out of the other variables, age was found to be the only significant coefficient in the

regression model. However, obesity, smoking status, diabetes status, gender, and cholesterol status were found to not be significant coefficients of the regression model.

The regression model was tested for high school graduates, representing individuals who have graduated high school or who have at least acquired a GED. A significant regression was found ($F(10,20) = 27.170$, $p < .001$), with the model explaining 63% (Nagelkerke R^2) of the variance and correctly classifying 97% of the cases for all variables in the model. Individuals with elevated homocysteine were 7.12 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 1.96$, $t(29) = 2.52$, $p < .05$, was seen to be a significant coefficient in the regression model. Additionally, age, obesity, and diabetes status were found to be significant coefficients in the regression model. However, smoking status, cholesterol status, and gender were found to not be significant coefficients of the regression model.

Finally, the overall regression was tested for individuals with at least some college education, representing the educated stratum of society. A significant regression was found ($F(10,21) = 12.274$, $p < .001$), with the model explaining 49% (Nagelkerke R^2) of the variance and correctly classifying 98% of the cases for all variables in the model. Individuals with elevated homocysteine were 3.83 times more likely to exhibit CRS than those without CRS. Elevated Hcy, $\beta = 1.34$, $t(30) = 3.10$, $p < .05$, was seen to be a significant coefficient in the regression model. Additionally, age, obesity, and diabetes status were found to be significant coefficients in the regression model. However, smoking status, cholesterol status, and gender were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that education level plays an effect modifying role on how homocysteine levels affect CRS as hypothesized. When comparing individuals who are high school graduates with individuals have at least some college education, individuals with a high school education have a much stronger association with CRS than those with at least some college education (OR 7.12 vs 3.83) as seen in Table 28 and Figure 23. Additionally, in every model tested in the high school graduate category, the odds ratio of elevated homocysteine on CRS was higher in individuals at the low education level. Results were inconsistent in individuals with only some high school. This may have occurred due to a smaller sample size.

Table 29

Age- and Sex-Adjusted Fibrinogen ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by ethnicity.

	Hispanic OR (95% CI) (N=532)	**Non-Hispanic White OR (95% CI) (N=1,280)**	**Non-Hispanic Black OR (95% CI) (N=417)**
Model 1 (Fibrinogen)	0.31 (0.09-1.07)	1.53 (0.88-2.65)	3.71 (1.45-9.51)*
Model 2 (Fibrinogen, obesity)	0.29 (0.07-1.31)	1.35 (0.65-2.78)	3.44 (1.44-8.23)*
Model 3 (Fibrinogen, obesity, diabetes status)	0.30 (0.06-1.42)	1.37 (0.68-2.75)	3.27 (1.15-9.36)*
Model 4 (Fibrinogen, obesity, diabetes status, smoking status)	0.42 (0.08-2.20)	1.30 (0.65-2.62)	3.04 (1.04-8.92)*
Model 5 (Fibrinogen, obesity, diabetes status, smoking status, hypercholesterolemia)	1.81 (.005-639.8)	0.96 (0.37-2.49)	8.53 (0.89-82.19)

Note. $*p < .05$

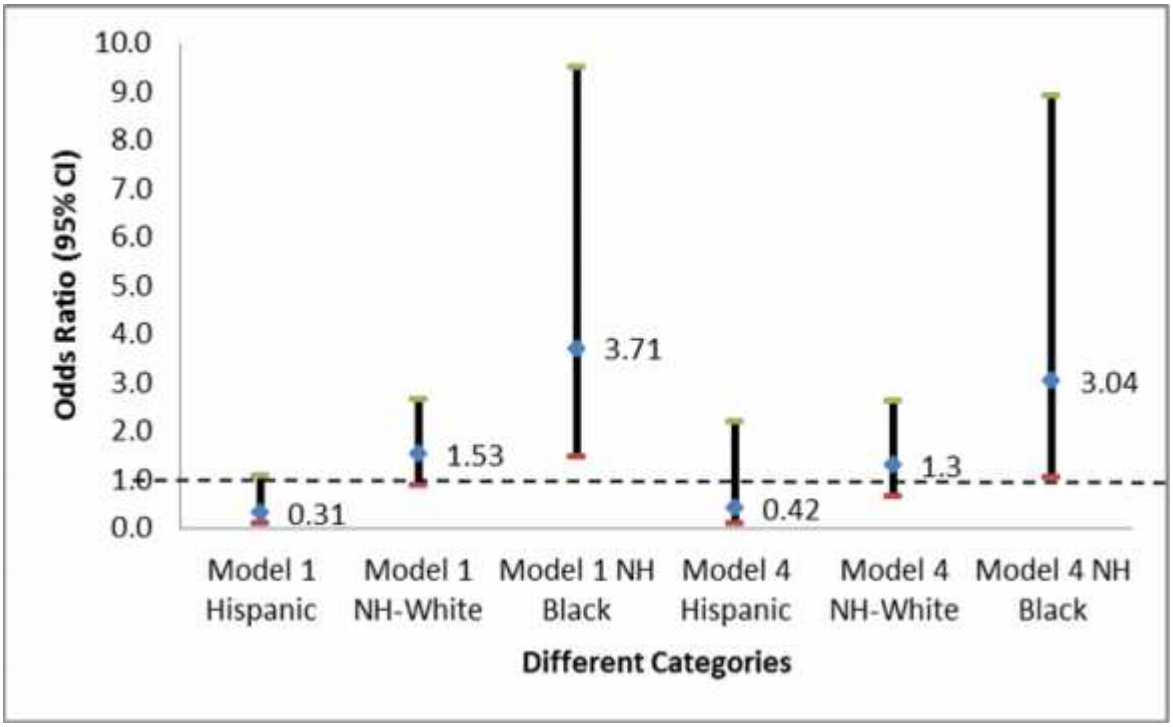

Figure 24. Effect of race on the relationship between fibrinogen and CRS before and after controlling for CRS risk factors

Initially, the total sample was stratified to assess for effect modification by race as seen in Table 29 and Figure 24. The first group of regression models was calculated for Hispanic individuals. A significant regression was found ($F(7, 2) = 87.533$, $p < .05$), with the model explaining 62% (Nagelkerke R^2) of the variance and correctly classifying 98% of the cases for all variables in the model. Individuals with elevated fibrinogen were 1.81 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 0.59$, $t(8) = 0.23$, $p = 0.82$, was seen to be a non-significant coefficient in the regression model. Additionally, age and cholesterol status were found to be the only significant

coefficients in the regression model. However, smoking status, gender, diabetes status, and obesity were found to not be significant coefficients of the regression model.

The regression model was tested for Non-Hispanic Whites. A significant regression was found ($F(10,6) = 1762.953$, $p < .001$), with the model explaining 55% (Nagelkerke R^2) of the variance and correctly classifying 93% of the cases for all variables in the model. Individuals with elevated hs-CRP were 0.96 times more likely to exhibit CRS than those without CRS. Elevated hs-CRP, $\beta = -0.04$, $t(15) = -0.08$, $p = 0.94$, was seen to be a non-significant coefficient in the regression model. Additionally, age, smoking status, and cholesterol status were found to be significant coefficients in the regression model. However, gender, diabetes status, and obesity were found to not be significant coefficients of the regression model.

The regression model was tested for Non-Hispanic Blacks. A significant regression was found ($F(8,1) = 2421.232$, $p < .05$), with the model explaining 58% (Nagelkerke R^2) of the variance and correctly classifying 90% of the cases for all variables in the model. Individuals with elevated fibrinogen were 8.53 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 2.14$, $t(8) = 2.18$, $p = 0.06$, was seen to be a non-significant coefficient in the regression model. Additionally, age, obesity, and cholesterol status were found to be significant coefficients in the regression model. However, diabetes status, smoking status, and gender were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that ethnicity plays an effect modifying role on how fibrinogen levels affect CRS as hypothesized. While in the

Non-Hispanic White and Hispanic groups, the odds of CRS in individuals with high fibrinogen was close to 1, in the non-Hispanic Black the odds ratio of CRS was much higher than 1 and statistically significant with most of the covariates as seen in Table 29 and Figure 24. The non-Hispanic Black population had the highest odds ratio at 8.53 when controlling for other CVD risk factors. Additionally, different cofactors were significant in each ethnic subpopulation.

Table 30

Age- and Sex-Adjusted Fibrinogen ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by income level.

	Total OR (95% CI) (N=2,124)	**Below 200% Poverty (95% CI) (N=746)**	**≥ 200% Poverty (95% CI) (N=1,378)**
Model 1 (Fibrinogen)	1.50 (0.83-2.69)	1.17 (0.60-2.26)	1.56 (0.62-3.92)
Model 2 (Fibrinogen, obesity)	1.31 (0.60-2.86)	1.02 (0.45-2.31)	1.42 (0.50-4.01)
Model 3 (Fibrinogen, obesity, diabetes status)	1.30 (0.60-2.81)	1.02 (0.45-2.31)	1.44 (0.52-4.04)
Model 4 (Fibrinogen, obesity, diabetes status, smoking status)	1.29 (0.60-2.80)	1.04 (0.43-2.50)	1.49 (0.52-4.27)
Model 5 (Fibrinogen, obesity, diabetes status, smoking status, hypercholesterolemia)	1.50 (0.58-3.91)	1.96 (0.60-6.42)	1.16(0.29-4.59)

Note. $*p < .05$ $**p < .001$

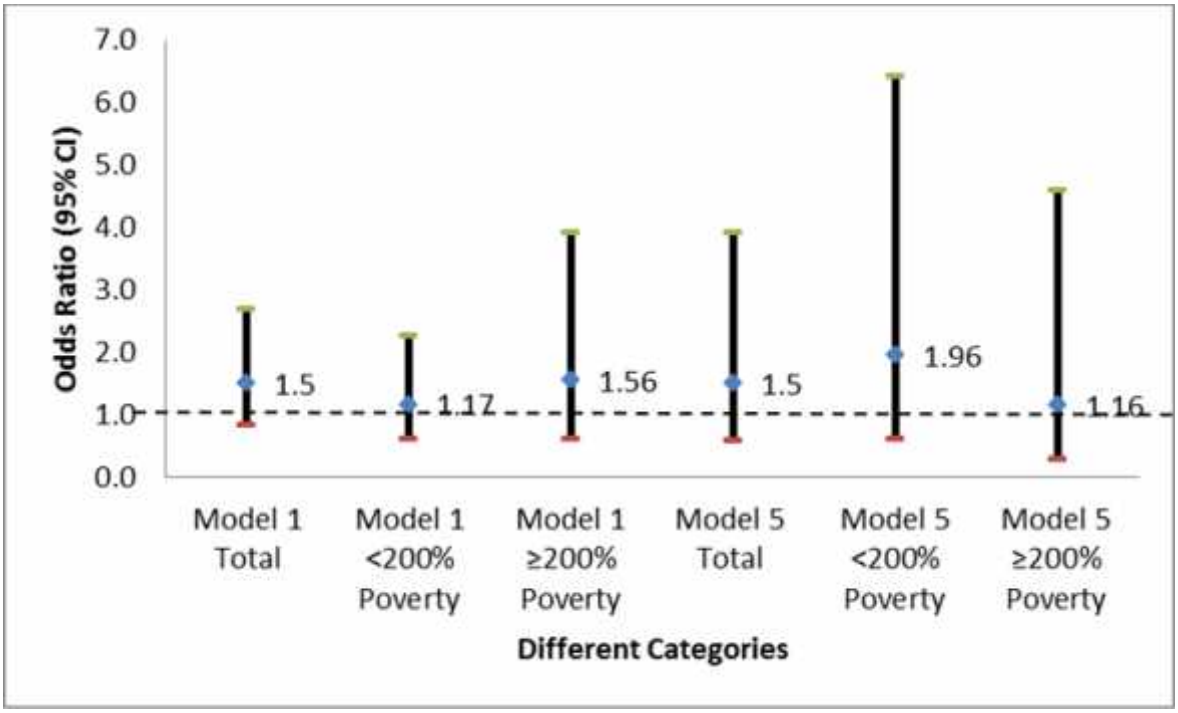

Figure 25: Effect of income level on the relationship between Fibrinogen and CRS before and after controlling for CRS risk factors.

Initially, the overall regression model was tested to determine how elevated fibrinogen played a role on the total sample as seen in Table 30 and Figure 25. A significant regression was found ($F(10,6) = 62.232$, $p < .001$), with the model explaining 50% (Nagelkerke R^2) of the variance and correctly classifying 93% of the cases for all variables in the model. Individuals with elevated fibrinogen were 1.50 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 0.41$, $t(15) = 0.90$, $p = 0.38$, was seen to be a non-significant coefficient in the regression model. Additionally, age, smoking status, and cholesterol status were found to be significant coefficients of the regression model. Gender and obesity were the only variables found to be not significant in the model.

The total sample was stratified to assess for effect modification. The regression model was tested at less than 200% of federal poverty levels, representing individuals in a low socioeconomic status. A significant regression was found ($F(10,5) = 7.455$, $p < .05$), with the model explaining 47% (Nagelkerke R^2) of the variance and correctly classifying 85% of the cases for all variables in the model. Individuals with elevated fibrinogen were 1.96 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 0.68$, $t(14) = 1.22$, $p=0.24$, was seen to be a non-significant coefficient in the regression model. Additionally, age was found to be significant coefficients in the regression model. However, smoking status, obesity, cholesterol status, diabetes status, and gender were found to not be significant coefficients of the regression model.

The overall regression model was tested at ≥ 200% of federal poverty levels, representing normal to high socioeconomic status. A significant regression was found ($F(10,6) = 1264.317$, $p < .001$), with the model explaining 54% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with elevated fibrinogen were 1.16 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 0.15$, $t(15) = 0.23$, $p = .82$, was seen to be a non-significant coefficient in the regression model. Additionally, age and cholesterol status were found to be significant coefficients of the regression model. However, obesity, gender, smoking status, and diabetes status was not found to be significant coefficients in the regression model.

Comparison of the aforementioned models demonstrates that income level does not play an effect modifying role on how fibrinogen levels affect CRS as hypothesized.

While in all three categories, gender was not a significant variable, when stratified by socioeconomic status, smoking status was not significant as well as seen in Table 30 and Figure 25. In every model in the low SES category, the odds ratio and confidence intervals of elevated fibrinogen on CRS was no different in individuals at any income level. Different variables were significant in each regression model.

Table 31

Age- and Sex-Adjusted Fibrinogen ORs for CRS in Multivariate Regression Modeling controlling for different CRS risk factors stratified by income level.

	Less than High School Graduate OR (95% CI) (N=343)	**High School Graduate (95% CI) (N=510)**	**At least Some College (95% CI) (N=1,102)**
Model 1 (Fibrinogen)	2.16 (0.67-6.94)	1.84 (0.57-5.93)	1.35 (0.67-2.72)
Model 2 (Fibrinogen, obesity)	1.74 (0.44-6.83)	1.43 (0.34-5.95)	1.33 (0.61-2.89)
Model 3 (Fibrinogen, obesity, diabetes status)	1.62 (0.39-6.72)	1.63 (0.37-7.10)	1.33 (0.60-2.95)
Model 4 (Fibrinogen, obesity, diabetes status, smoking status)	1.85 (0.47-7.25)	1.53 (0.36-6.51)	1.36 (0.60-3.11)
Model 5 (Fibrinogen, obesity, diabetes status, smoking status, hypercholesterolemia)	3.84 (0.51-29.04)	2.07 (0.43-10.01)	0.71 (0.19-2.58)

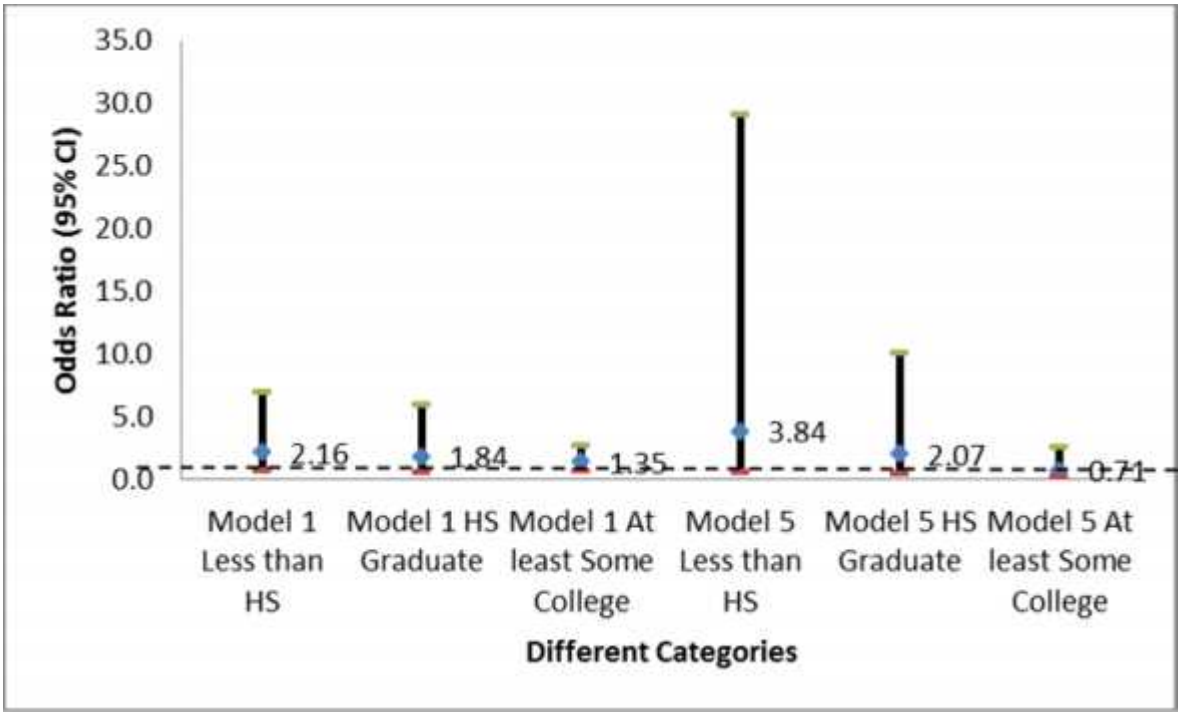

Figure 26: Effect of Education Level on the relationship between Fibrinogen and CRS before and after controlling for CRS risk factors

The total sample was stratified by education level to assess for effect modification as seen in Table 31 and Figure 26. Initially, the overall regression model was tested to determine how elevated fibrinogen played a role on individuals with less than a high school education. A significant regression was found ($F(10,6) = 2144.229$, $p < .001$), with the model explaining 49% (Nagelkerke R^2) of the variance and correctly classifying 79% of the cases for all variables in the model. Individuals with elevated fibrinogen were 3.84 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 1.35$, $t(15) = 1.42$, $p = 0.18$, was seen to be a non-significant coefficient in the regression model. Out of the other variables, age and cholesterol status were found to be the only significant coefficient in the regression model. However, smoking status, obesity,

diabetes status, and gender were found to not be significant coefficients of the regression model.

The regression model was tested for high school graduates, representing individuals who have graduated high school or who have at least acquired a GED. A significant regression was found ($F(10,6) = 89.109$, $p < .001$), with the model explaining 59% (Nagelkerke R^2) of the variance and correctly classifying 94% of the cases for all variables in the model. Individuals with elevated fibrinogen were 2.07 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = 0.73$, $t(15) = 0.98$, $p = 0.34$, was seen to be a non-significant coefficient in the regression model. Additionally, age and cholesterol status were found to be significant coefficients in the regression model. However, gender, smoking status, diabetes status, and obesity were found to not be significant coefficients of the regression model.

Finally, the overall regression was tested for individuals with at least some college education, representing the educated stratum of society. A significant regression was found ($F(9,7) = 399.130$, $p < .001$), with the model explaining 53% (Nagelkerke R^2) of the variance and correctly classifying 95% of the cases for all variables in the model. Individuals with elevated fibrinogen were 0.71 times more likely to exhibit CRS than those without CRS. Elevated fibrinogen, $\beta = -0.35$, $t(15) = -0.57$, $p = 0.58$, was seen to be a non-significant coefficient in the regression model. Additionally, age and obesity were found to be significant coefficients in the regression model. However, smoking status, cholesterol status, diabetes status, and gender were found to not be significant coefficients of the regression model.

Comparison of the aforementioned models demonstrates that education level plays an effect modifying role on how fibrinogen levels affect CRS as hypothesized. When comparing individuals with at most a high school education with individuals with more than a high school education, individuals with more than a high school education have an odds ratio close to 1 as seen in Table 31 and Figure 26. Additionally, in every model tested in the high school graduate category, the odds ratio of elevated fibrinogen on CRS was higher in individuals with only a high school education. The covariates that were significant were different in each model.

Summary of Results

In summary, when tested for additive effect of each inflammatory marker (hs-CRP, homocysteine, fibrinogen, and F/T Ratio) on CRS after controlling for known risk factors, there was a significant effect found for hs-CRP, homocysteine, and fibrinogen. However, for F/T ratio there was a non-significant additive effect, when controlling for other risk factors. Additionally, there was a significant modifying effect by hs-CRP, homocysteine, and fibrinogen in the context of Type 4 and Type 2 CRS even after controlling known CVD and CKD risk factors. However, due to the lack of adequate subjects, the modifying effect of F/T ratio could not be tested. Finally, in the context of demographic factors, race modified the effect of inflammatory markers on CRS. Income modified the effect of inflammatory markers on CRS, except in the context of hs-CRP and fibrinogen. Education level also modified the effect of inflammatory markers on CRS. However, due to the lack of adequate subjects, the modifying effect of F/T ratio could not be tested.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

Through the analysis of NHANES data, 1999-2010, the theory was evaluated that if elevated levels of biomarkers in the context of the development of cardiovascular disease leads to the subsequent development of chronic kidney disease or vice versa among a multi-ethnic high risk population group. Through analysis of the same NHANES data sets, the theory was also evaluated if education level, income level, and race modify the relationship between inflammatory biomarkers and CRS. CRS has been shown to be present in relationship with heart failure and other chronic conditions. For instance, as many as one-third of individuals who have acute decompensated heart failure have been shown to have type 1 CRS (Ronco et al., 2010). From a pathophysiological perspective, diabetes, obesity, hypertension, smoking status, elevated serum cholesterol levels, and even elevated inflammatory markers have been shown to have a close interrelationship with cardiovascular disease and chronic kidney disease individually (Levitan et al., 2009; Liu et al., 2012; Nguyen et al., 2009). However, not much research has been done in studying how to prevent CRS (Lekawanvijit et al., 2012). Understanding the pathophysiology of specific cardiovascular disease (i.e. CHF) and renal disease like can lead to identifying critical biomarkers that could signal clinically aberrant manifestations before they occur (Xue, Chan, Sakariya, & Maisel, 2010). Individuals in the U.S. who have a history of diabetes, obesity, have elevated serum cholesterol levels, and have a positive smoking status also have an increased risk for cardiovascular disease. The results from this study concluded that elevated serum

inflammatory marker levels independently and additively predict CRS in individuals in the United States. Positive social change is instrumental for the implementation of revised policies and protocols, creation of novel funding mechanisms, and development of specific intervention programs aimed at educating about CRS and preventing rapid progression of CRS in individuals, ages 20 years and older in the U.S. population.

Interpretation of Findings

According to the literature, diabetes, hypertension, obesity, and increased serum cholesterol were all shown to increase the risk of cardiovascular disease and cardiorenal syndrome in U.S. individuals, aged 20 years and older (Levitan et al., 2009; Liu et al., 2012). In all individuals, inflammatory biomarkers were also shown to increase the risk of cardiovascular disease. The literature also provided evidence that obesity and hypertension cause elevated serum biomarkers like hs-CRP and homocysteine levels. In the current study, data from NHANES, 1999-2010, showed that hs-CRP levels, fibrinogen, and homocysteine have an additive effect on CRS and independently modifies the effect in in the U.S. population. Additionally, demographic factors modify the effect of hs-CRP, fibrinogen and hcy on CRS in the U.S. population except for income level on hs-CRP. More data was necessary in order to study the effect of F/T ratio.

Population-Specific CRS Research Findings

Data was available from NHANES, 1999-2010, on 1,548 individuals with CRS, aged 20-85 years. The majority of the population was non-Hispanic White women, with a mean age 72.8 years. There were proportional numbers of non-Hispanic blacks and Hispanics to non-Hispanic Whites. There were also proportional numbers of individuals

aged 20-34 years and 66-85 years, when compared to those aged 35-65 years. The category of multiracial and other races were lacking in the study population. Nearly 40% of all study participants had some college education or were college graduates.

Overall, serum hs-CRP and cholesterol levels were elevated for the majority of the study population. Overweight/obesity and hypertension were both highly prevalent in the study population. The incidence of cardiovascular disease was quite low because, according to self-reported data, very few individuals reported having physician-diagnosed heart attack, coronary heart disease, congestive heart failure, and angina. Concurrently, very few individuals had GFR less than 60, as calculated through the Cockcroft-Gaullt equation from measured creatinine. Thus, the incidence of CKD was quite low and CRS was even lower.

Hypothesis 1

Hypothesis 1 predicted that elevated inflammatory biomarker levels modify the effect of CKD on cardiovascular health in the U.S. population after controlling for obesity, hypercholesterolemia, diabetes status, and smoking status. Demographic variables including age and gender were also controlled for to prevent potential confounders. Twelve sets of separate multiple regression analyses were performed. For hs-CRP, hcy, and fibrinogen the null hypothesis was rejected in lieu of the alternative hypothesis that the elevated inflammatory biomarkers do modify the effect of CKD on CVD controlling for CVD and CKD risk factors. Due to the lack of adequate subjects, the modifying effect of F/T ratio could not be tested, and the null hypothesis could not be rejected.

The first set of multiple regression analyses set were performed to determine if elevated serum hs-CRP levels after controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status modify the effect of CVD on CKD in a multi-ethnic population aged 20 years and older within the U.S. population. Results showed that elevated serum hs-CRP levels modifies the effect of CVD for predicting stage 3 CKD and higher in individuals aged 20 years and older in the U.S. population. Obesity, hypercholesterolemia, diabetes status, and smoking status were significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hs-CRP levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The second set of multiple regression analyses set were performed to determine if elevated serum homocysteine levels after controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status modify the effect of CVD on CKD in a multi-ethnic population aged 20 years and older within the U.S. population. Results showed that elevated serum hcy levels modifies the effect of CVD for predicting stage 3 CKD and higher in individuals aged 20 years and older in the U.S. population. Obesity, hypercholesterolemia, diabetes status, and smoking status were significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hcy levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The third set of multiple regression analyses set were performed to determine if elevated serum fibrinogen levels after controlling for obesity, age, hypercholesterolemia,

gender, diabetes status, and smoking status modify the effect of CVD on CKD in a multi-ethnic population aged 20 years and older within the U.S. population. Results showed that elevated serum fibrinogen levels modifies the effect of CVD for predicting stage 3 CKD and higher in individuals aged 20 years and older in the U.S. population. Obesity, hypercholesterolemia, diabetes status, and smoking status were significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum fibrinogen levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

Hypothesis 2

Hypothesis 2 predicted elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) modify the effect of CVD on CKD after controlling for CVD and CKD risk factors like obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. For this analysis, four sets of multiple regression analyses were performed. For hs-CRP, hcy, and fibrinogen the null hypothesis was rejected in lieu of the alternative hypothesis that the elevated inflammatory biomarkers do modify the effect of CVD on CKD controlling for CVD and CKD risk factors. There was not enough data to adequately complete the analysis to assess the modifying effect of F/T ratio, and the null hypothesis could not be rejected.

The first set of multiple regression analyses set were performed to determine if elevated serum hs-CRP levels controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status modify the effect of CKD on CVD in a multi-ethnic population aged 20 years and older within the U.S. population. Results showed that

elevated serum hs-CRP levels modifies the effect of CKD for predicting cardiovascular health in individuals aged 20 years and older in the U.S. population. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hs-CRP levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The second set of multiple regression analyses set were performed to determine if elevated serum homocysteine levels controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status modify the effect of CKD on CVD in a multi-ethnic population aged 20 years and older within the U.S. population. Results showed that elevated serum hcy levels modifies the effect of CKD for predicting cardiovascular health in individuals aged 20 years and older in the U.S. population. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hcy levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The third set of multiple regression analyses set were performed to determine if elevated serum fibrinogen levels controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status modify the effect of CKD on CVD in a multi-ethnic population aged 20 years and older within the U.S. population. Results showed that elevated serum fibrinogen levels modifies the effect of CKD for predicting cardiovascular health in individuals aged 20 years and older in the U.S. population.

Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum fibrinogen levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

Hypothesis 3

The third set of multiple regression analyses were performed to determine if elevated serum elevated inflammatory biomarkers (hcy, F/T, fibrinogen, and hs-CRP) have an additive effect on CRS along with CVD risk factors like obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. For all of the inflammatory biomarkers, the null hypothesis was rejected in lieu of the alternative hypothesis that elevated specific inflammatory biomarkers act as additive risk factors and increase the susceptibility of CRS along with known CVD risk factors.

Results showed that elevated serum ethnicity levels along with the additive effect of obesity, cholesterol status, smoking status, diabetes status predict cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. While gender was non-significant, the demographic variable of age had an additive effect along with elevated serum hs-CRP levels in predicting CRS in individuals aged 20 years and older in the U.S. population.

Furthermore, results showed that elevated serum hcy levels when controlling for obesity, cholesterol status, smoking status, diabetes status predict cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. Obesity, cholesterol status, smoking status, and diabetes status were non-significant predictors for CRS. While

gender was non-significant, the demographic variable of age had an additive effect along with elevated serum hcy levels in predicting CRS in individuals aged 20 years and older in the U.S. population.

Results showed that elevated serum fibrinogen levels along with the additive effect of obesity, cholesterol status, smoking status, diabetes status predict cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. Obesity, cholesterol status, smoking status, and diabetes status were non-significant predictors for CRS. While gender was non-significant, the demographic variable of age had an additive effect along with elevated serum fibronogen levels in predicting CRS in individuals aged 20 years and older in the U.S. population. While gender was non-significant, the demographic variable of age had an additive effect along with elevated serum fibrinogen levels in predicting CRS in individuals aged 20 years and older in the U.S. population.

Finally, results showed that elevated serum F/T ratio levels along with the additive effect of obesity, cholesterol status, smoking status, diabetes status non-significantly predict cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. Diabetes was the only medical variable that was significant in this model. Individuals with elevated F/T ratio had a 31% higher chance of having CRS than those without elevated CRS. While gender was not considered because only females were in this sample, the demographic variable of age had an additive effect in predicting CRS in individuals aged 20 years and older in the U.S. population.

Hypothesis 4

The fourth set of multiple regression analyses were performed to determine if sociodemographic (race/ethnicity, family income, expressed relative to the poverty threshold, or education level) indicators play a modifying role between the relationship of inflammatory markers and CRS after controlling for known CRS risk factors like obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. In the context of race, the null hypothesis was rejected in lieu of the alternative hypothesis that race plays a modifying role between the relationship of inflammatory markers and CRS. However, for income, only one inflammatory the null hypothesis was rejected only in the context of one biomarker, hcy. The null hypothesis was rejected in lieu of the alternative hypothesis that income played a modifying role between the relationship of hcy and CRS . For hs-CRP and fibrinogen, the null hypothesis was not rejected since income failed to play a modifying role between the relationship of hs-CRP and fibrinogen and CRS. Finally, due to the lack of adequate subjects, the modifying effect of demographic factors could not be tested between F/T ratio and cardiorenal syndrome.

The first set of multiple regression analyses set were performed to determine if ethnicity modify the effect of hs-CRP on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that ethnicity modifies the effect of hs-CRP for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. While in the Non-Hispanic White, Non-Hispanic Black, and Hispanic populations the odds ratio of CRS was much higher than 1 and statistically

significant, the Non-Hispanic White population had the lowest odds ratio at 2.26. The Hispanic population had the highest odds ratio at 4.31 when controlling for other CVD risk factors. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hs-CRP levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The second set of multiple regression analyses set were performed to determine if income level modify the effect of hs-CRP on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that income level does not modify the effect of hs-CRP for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. In every model in the low SES category, the odds ratio and confidence intervals of elevated hs-CRP on CRS was lower in individuals at the high income level. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hs-CRP levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The third set of multiple regression analyses set were performed to determine if education level modify the effect of hs-CRP on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age,

hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that education level modifies the effect of hs-CRP for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. In every model in the low SES category, the odds ratio and confidence intervals of elevated hs-CRP on CRS was lower in individuals at the high education level. When comparing individuals with at most a high school education with individuals with more than a high school education, individuals with more than a high school education have an odds ratio close to 1. Additionally, in every model tested in the high school graduate category, the odds ratio of elevated hs-CRP on CRS was higher in individuals with only a high school education. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hs-CRP levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The fourth set of multiple regression analyses set were performed to determine if ethnicity modify the effect of hcy on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that ethnicity modifies the effect of hcy for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. While in the Non-Hispanic White and Non-Hispanic Black the odds ratio of CRS were much higher than 1, in the Hispanic population the odds ratio was close to 1. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables

(i.e., age and gender) had an additive effect along with elevated serum hcy levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The fifth set of multiple regression analyses set were performed to determine if income level modify the effect of hcy on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that income level does modify the effect of hcy for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. While in the overall model and ≥ 200% of federal poverty levels only diabetes status and age played a role in affecting CRS, in the lower socioeconomic status group only, cholesterol status and obesity played a role. Additionally, in every model in the low SES category, the odds ratio and confidence intervals of elevated homocysteine on CRS were higher in individuals at the low income level. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hcy levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The sixth set of multiple regression analyses set were performed to determine if education level modify the effect of hcy on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that

education level modifies the effect of hcy for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population When comparing individuals who are high school graduates with individuals have at least some college education, individuals with a high school education have a much stronger association with CRS than those with at least some college education (OR 7.12 vs 3.83). Additionally, in every model tested in the high school graduate category, the odds ratio of elevated homocysteine on CRS was higher in individuals at the low education level. Results were inconsistent in individuals with only some high school. This may have occurred due to a smaller sample size. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum hs-CRP levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The seventh set of multiple regression analyses set were performed to determine if ethnicity modify the effect of fibrinogen on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that ethnicity modifies the effect of fibrinogen for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. While in the Non-Hispanic White and Hispanic groups, the odds of CRS in individuals with high fibrinogen was close to 1, in the non-Hispanic Black the odds ratio of CRS was much higher than 1 and statistically significant with most of the covariates. The non-Hispanic Black population

had the highest odds ratio at 8.53 when controlling for other CVD risk factors. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum fibrinogen levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The eighth set of multiple regression analyses set were performed to determine if income level modify the effect of fibrinogen on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that income level does not modify the effect of fibrinogen for predicting cardiorenal syndrome in individuals aged 20 years and older in the U.S. population. In every model in the low SES category, the odds ratio and confidence intervals of elevated fibrinogen on CRS was no different in individuals at any income level. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum fibrinogen levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

The ninth set of multiple regression analyses set were performed to determine if education level modify the effect of fibrinogen on CRS in a multi-ethnic population aged 20 years and older within the U.S. population controlling for obesity, age, hypercholesterolemia, gender, diabetes status, and smoking status. Results showed that education level modifies the effect of fibrinogen for predicting cardiorenal syndrome in

individuals aged 20 years and older in the U.S. population. When comparing individuals with at only a high school education with individuals with more than a high school education, individuals with more than a high school education have an odds ratio close to 1. Additionally, in every model tested in the high school graduate category, the odds ratio of elevated fibrinogen on CRS was higher in individuals with only a high school education. Obesity, hypercholesterolemia, diabetes status, and smoking status were differentially significant in the regression models. In addition, demographic variables (i.e., age and gender) had an additive effect along with elevated serum fibrinogen levels in predicting cardiovascular disease in individuals aged 20 years and older in the U.S. population.

Summation of Findings

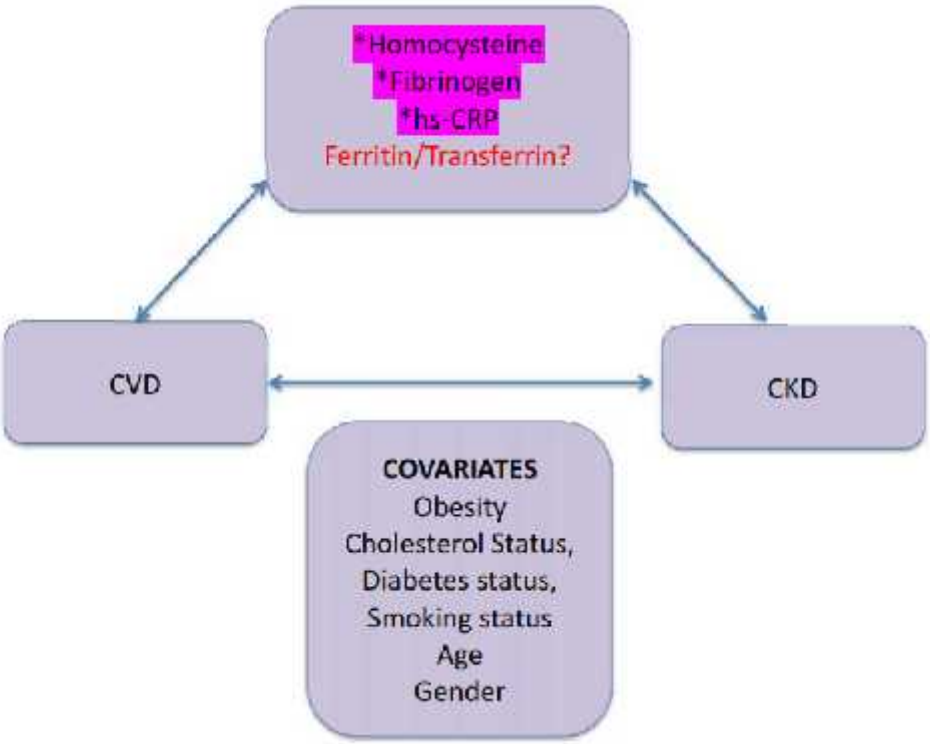

Figure 27. Inflammatory biomarkers involved in effect modification of Type 2 CRS and Type 4 CRS.

Overall, when tested for additive effect of each inflammatory marker (hs-CRP, homocysteine, fibrinogen, and F/T Ratio) on CRS after controlling for known risk factors, there was a significant effect found for hs-CRP, homocysteine, and fibrinogen. However, for F/T ratio there was a non-significant additive effect, when controlling for other risk factors. Additionally, there was a significant modifying effect by hs-CRP, homocysteine, and fibrinogen in the context of Type 4 and Type 2 CRS even after controlling known CVD and CKD risk factors as shown by the highlighted biomarkers in Figure 27. However, due to the lack of adequate subjects, the modifying effect of F/T

ratio could not be tested, which is denoted by ferritin/transferrin written in red. Finally, in the context of demographic factors, race modified the effect of inflammatory markers on CRS. Income modified the effect of inflammatory markers on CRS, except in the context of hs-CRP and fibrinogen. Education level also modified the effect of inflammatory markers on CRS. However, due to the lack of adequate subjects, the modifying effect of F/T ratio could not be tested.

Implications for Social Change

The results of the current study suggest both direct and indirect sources of positive social change. On a direct level, the findings from this study provide evidence of a direct link between tangible and identifiable biomarkers and CRS. This can lead to dissemination of information to promote discussion in conferences and medical communities in order to increase awareness. On an indirect level, findings from this study can lead to positive social change to control cardiorenal syndrome in individuals, aged 20 years and older, in the United States: implementing new policies and procedures aimed at health and nutrition awareness, creating new funding mechanisms, and developing intervention programs aimed at controlling CRS.

The finding from this study will aid in creating positive social change by providing evidence that will be useful for developing and implementing new policies for control of cardiovascular disease and cardiorenal syndrome. While the Cardiorenal Society of America has teamed up with the National Kidney Foundation to disseminate information about CRS, more information needs to reach physicians and the public about the unique challenges surrounding CRS and the nutritional changes that are necessary.

More funding is necessary in order educate public health practitioners about the deleterious effects of inflammation. New policies and procedures aimed at health and nutrition awareness provided additional federal and state funding for intervention programs aimed at combating obesity, which both helped reduce elevated serum inflammatory markers and cholesterol levels. Implementation of intervention programs will allow improvement of quality of life and prevention of cardiorenal syndrome.

Monitoring C-reactive protein and other inflammatory markers is another change in clinical practice that can save money and lives in the future. Additionally, making this a national goal like through Healthy People 2020, can let patients and health care providers realize the importance of inflammation. Obesity has clearly been shown to be associated with inflammation. With this being a common risk factor for many different conditions, it is also important to study this connection in the context of CRS. While Healthy People 2020 has cardiovascular related goals, there are no goals specifically related to inflammation. The challenge is to make a case strong enough to convince public policy makers to invest money into doing research and implement public health initiatives in this area.

Recommendations for Action

The study findings reiterate how imperative it is to increase awareness and promote healthy living by controlling and preventing chronic kidney disease, cardiovascular disease, and cardiorenal syndrome. Because the link between inflammation and CRS has been established, now the next step is to empower patients through knowledge of cardiorenal disease likelihood in order to achieve greater health

equity in populations with greater barriers to access. As demonstrated by Mogford, Gould, and Devoght (2011), by motivating, engaging, and empowering individuals on specific health topics like the importance of inflammation in disease progression, improvements can be made in physical health in high risk groups. Through this dissertation, the assertion may be made for a greater awareness of the role of inflammation in disease progression which may help both health practitioners and patients alike. In this paradigm shift, the crux of medical practice needs to shift from treating each individual organ to treating the body as a whole (Wallace & Wallace, 2004). In cardiovascular disease, health practitioners are beginning to recognize the dire need to track inflammatory markers. For instance, Kaptoge et al. (2012) found from a meta-analysis of 52 studies that if inflammatory biomarkers like fibrinogen and hs-CRP are measured in those deemed to have intermediate risk of cardiovascular disease, then a considerable amount of subsequent cardiovascular events can be decreased. Similarly, the importance of inflammatory markers in the development of subsequent renal disease needs to be emphasized among patients and become a national priority (Stenvinkel, 2010).

The implications from this study are multifold. The findings from this study will not only impact individuals who have CRS, but also bring a general awareness about the importance of monitoring inflammatory markers in CKD and even CVD. Dieticians and nutritionists can utilize this information to educate individuals about following an anti-inflammatory diet which leads to optimal health. Nutrition labels can go as far as to indicate the dietary inflammatory index of specific food to indicate to consumers directly

which food causes an increase in inflammation and which do not. For implementation of these policies, major governmental agencies like the Food and Drug Administration need to be convinced of the importance on proper anti-inflammatory nutrition in the context of cardiovascular disease and cardiorenal syndrome. Additionally, taking certain vitamins and dietary supplements could aid in decreasing inflammation and preventing the progression and development of CRS. By providing knowledge to healthcare providers and patients, they will be empowered to make positive changes in their life. Prevention of disease will lead to lower health care related expenditures and propagation of positive social change from health care workers to patients.

Limitations and Recommendations for Further Study

There were multiple limitations in this study that will need to be adequately addressed. There is insufficient preexisting information concerning the association of inflammatory markers and CRS. Another related limitation was the inability to extrapolate association data to represent causation data. In order to overcome this lack of preexisting information, a methodical approach was employed where theoretical frameworks and conceptual models were utilized to better understand the association. By applying existing models to better understand the relationship, the etiology of CRS was better elucidated, making prevention a possible option. With the framework established in this study future studies can be performing by following a cohort longitudinally similar to the Framingham cohort and studying if increased inflammatory markers lead to CRS and CRS exacerbation. This will also help establish causation.

Another limitation was the presence of potentially large false positive rates in this study due to multiple hypothesis testing. Even though according to Rothman (1990), no adjustments are needed in multiple hypothesis testing and Cohen (1994) demonstrates that corrections increase Type II error rate, there is controversy surrounding testing of multiple hypotheses. In order to assess for underlying patterns and effect modification, correction was not used for this study. However, for future studies a feasible Type 1 error correction method like Bonferroni correction can be utilized in order to specifically measure the effect of a single biomarker in the context of several covariates. This method on the conservative end also has its drawbacks due to comparison of an omnibus alternative hypothesis against the backdrop of a generic and universal null hypothesis (Glickman, Rao, & Schultz, 2014). Another alternative calculation approach available through statistical packages is the Benjamin and Hochberg method for calculating the false discovery rate (Hu, Zhao, & Zhou, 2010). This addresses the presence of Type 1 error more effectively.

The third limitation is that when using previously collected survey data, there were limitations as to what types of questions and how they were asked. When using previously collected data, the problem of the increased statistical error may occur because of the lack of ability to check for complete accuracy of the data presented. Because the NHANES survey had gone through numerous iterations in the past four decades, researchers had made sure to include as many questions as are appropriate for researchers to analyze data for stakeholders. Additionally, due to the inability to check accuracy of data, there may be an increase in statistical error. As mentioned previously, because of the reputation for validity in collection and recording of the

data, this potential error is minimal. Any possible errors can also be mitigated by using overlapping information from so that data can be cross-checked. In future studies more use of overlapping data can overcome the potential for bias.

The effect modification method utilized in this study could be a source of potential limitations. Even though there are multiple ways to approach effect modification, in this study the stratified regression approach was utilized (Van Ness & Allore, 2003). While this approach reduces the effect of multicollinearilty and there is no test for statistical significance, the interpretation of this method is intuitive and thus widely used. For future studies, in order to overcome this shortcoming techniques like centering can be utilized. This allows for better generalization to the population. Additionally, other approaches like the analytic approach, interaction term approach, specified levels approach, nesting approach, and the centered interaction term approach can be utilized in future studies in order to assess for effect modification.

Another potential limitation is the reliance on self-reported information, which may lead to bias. Again, in order to address this limitation, objective, examination information was used. In order to make the dataset more robust, instead of utilizing self-reported data, future studies can be conducted with medical personnel collecting the data. However, this approach would compromise the national representativeness of the study.

While the NHANES dataset is compatible with specific programs, all functions are not available without the knowledge or access to specific programs. Consequently,

another limitation is that rudimentary knowledge in weighting methods and analysis in specific statistical software is necessary for the proper use of the data.

Finally, a limitation that is an aspect of many cross-sectional studies is that the biomarker levels are a reflection of one moment in time and could be as a result of a spurious factor like infection. In order to control for these factors a large, representative population was utilized for this study. For future studies, inflammatory biomarker information needs to be collected at several periods of time in order to reflect the trend. This type of data is only possible with the establishment of a registry or surveillance type of data collection method.

Summary

In summary hs-CRP, homocysteine, and fibrinogen had a modifying effect on Type 4 (chronic reno-cardiac etiology) and Type 2 CRS (chronic cardio-renal etiology) and a significant additive effect on CRS even after controlling known CVD and CKD risk factors. Secondarily, different demographic factors modify the effect of inflammatory biomarkers on CRS differentially. While F/T ratio had a non-significant additive effect, due to the lack of adequate subjects, the modifying effect of F/T ratio could not be tested. This knowledge has major implications for social change in that health care professionals and patients must give as much attention to traditional CVD risk factors as they do to inflammatory risk factors. Future longitudinal studies need to be done to assess the causative effect of inflammatory biomarkers.

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Appendix A: IRB Approval

Dear Mr. Banerjee,

This email is to notify you that the Institutional Review Board (IRB) has approved your application for the study entitled, "Inflammatory Markers associated with Disease Progression of Cardiorenal Syndrome: An analysis of National Health and Nutritional Examination Survey."

Your approval # is 05-27-14-0303779. You will need to reference this number in your dissertation and in any future funding or publication submissions.

Your IRB approval expires on May 26, 2015. One month before this expiration date, you will be sent a Continuing Review Form, which must be submitted if you wish to collect data beyond the approval expiration date.

Your IRB approval is contingent upon your adherence to the exact procedures described in the final version of the IRB application document that has been submitted as of this date. This includes maintaining your current status with the university. Your IRB approval is only valid while you are an actively enrolled student at Walden University. If you need to take a leave of absence or are otherwise unable to remain actively enrolled, your IRB approval is suspended. Absolutely NO participant recruitment or data collection may occur while a student is not actively enrolled.

If you need to make any changes to your research staff or procedures, you must obtain IRB approval by submitting the IRB Request for Change in Procedures Form. You will receive confirmation with a status update of the request within 1 week of submitting the change request form and are not permitted to implement changes prior to

receiving approval. Please note that Walden University does not accept responsibility or liability for research activities conducted without the IRB's approval, and the University will not accept or grant credit for student work that fails to comply with the policies and procedures related to ethical standards in research.

When you submitted your IRB application, you made a commitment to communicate both discrete adverse events and general problems to the IRB within 1 week of their occurrence/realization. Failure to do so may result in invalidation of data, loss of academic credit, and/or loss of legal protections otherwise available to the researcher.

Both the Adverse Event Reporting form and Request for Change in Procedures form can be obtained at the IRB section of the Walden web site or by emailing irb@waldenu.edu:

http://researchcenter.waldenu.edu/Application-and-General-Materials.htm

Researchers are expected to keep detailed records of their research activities (i.e., participant log sheets, completed consent forms, etc.) for the same period of time they retain the original data. If, in the future, you require copies of the originally submitted IRB materials, you may request them from Institutional Review Board.

Sincerely,

Jenny Sherer, M.Ed., CIP

Associate Director

Office of Research Ethics and Compliance

Fax: 626-605-0472

Phone: 612-312-1341

Office address for Walden University:

100 Washington Avenue South

Suite 900

Appendix B: Permission Request and Correspondence

8/19/13

Srikanta Banerjee <srikanta.banerjee@waldenu.edu>

to cronco

Dr. Ronco,
I am a PhD student writing a dissertation on CRS. I reviewed many of your papers on CRS and was wondering if the cardiac component of Type 2 and Type 4 CRS can include different cardiovascular diseases like atrial fibrillation, stroke, and angina or does it just refer to CHF? Thank you.

Sincerely,
Srikanta Banerjee, MPH

8/20/13

Claudio Ronco <cronco@goldnet.it>

to me

Absolutely yes. These are most likely effects of a chronic disorder and so they are definitely part of the picture

Claudio Ronco, MD

Director
Dep. Nephrology Dialysis & Transplantation
International Renal Research Institute (IRRIV)
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Tel. Fax. +39 0444 753949
mailto: cronco@goldnet.it

Dr. McCullough
I am writing a PhD dissertation about cardiorenal syndrome at Walden University and found an excellent explanatory diagram that I wanted to use. Specifically may I use Figure 1: Pathophysiology and definitions of the five subtypes of cardiorenal syndromes from the paper cited below, for my dissertation? Please let me know. Thank you.

McCullough, P. (2010). Cardiorenal syndromes: pathophysiology to prevention. *International Journal Of Nephrology*, *2011*762590. doi:10.4061/2011/762590.

Srikanta Banerjee, MPH

10/18/13

Peter McCullough <peteramccullough@gmail.com>

to me

Permission granted